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Guest Editor

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1. Breneman, G. M., and Priest, E. McC.: Am. Heart J. 50:129 (July) 1955. 2. Tandowsky, R. M.: Am. J. Cardiol. 3:551 (April) 1959.

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1. Modell, W.: Am. J. Cardiol. 3:139 (Feb.) 1959.

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References: 1. Tandowsky, R. M.: Personal communication.
2. Parsons, W. B.: Curr. Therapeut. Res. 2 137 (May) 1960.
3. Thompson, C. E.: Personal communication.
4. Biben, L. H.; Kurstin, W., and Protas, M.: Personal communication.
5. Hobbs, T. G.: Personal communication.
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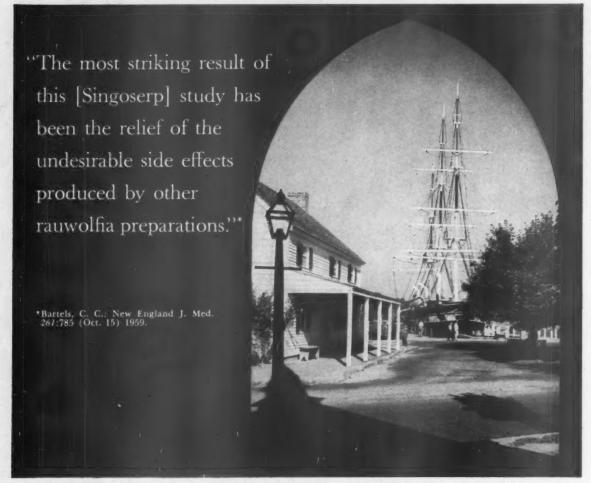
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PHILIP LISAN, WILBUR OAKS AND JOHN H. MOYER 246

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1. Biegeleisen, H. I.: Clin. Med. 2:1005, 1955. 2. Roberts, J. T.: Clin. Med. 4:1375, 1957.

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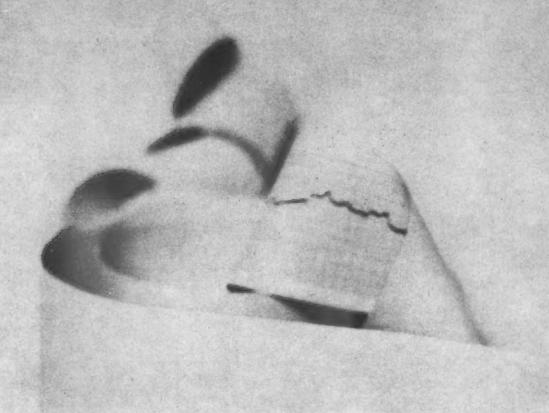
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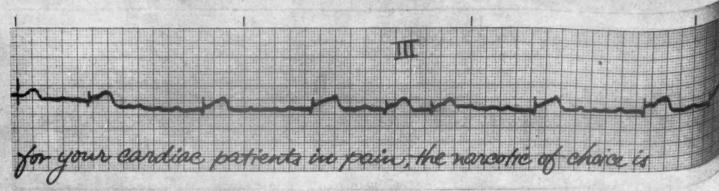
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The over-all clinical implications of manifest atherosclerotic disease in any one region of the cerebral bed

are therefore more serious than manifest clinical disease in a single coronary branch.





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Digitalis, Electrolytes and the Surgical Patient
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Vitally important in the management of surgical patients with heart disease is the knowledge of the complex interrelationships between electrolytes and digitalis action which the authors outline. In the digitalized patient a decrease in body potassium may precipitate digitalis intoxication. Potassium administration will abolish all digitalis-induced arrhythmias but toxic doses of digitalis interfere with the deposition of potassium within the body, probably releasing potassium by the liver and interfering with its uptake by skeletal muscle. Clinical case problems solved by tempering laboratory data with clinical acumen supplement experimental evidence and round out a useful, significant contribution.

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Besides presenting an eminent neurologist's observations on angina pectoris, this milestone should regenerate interest in electrotherapy either as a therapeutic method or as a device for extending our knowledge of the neural pathways involved in anginal attacks.

Case Reports

The clinical history and anatomic anomalies in an infant with isolated congenital absence of the right pulmonary artery are described. This case verified by autopsy is the fifth known. Unilateral pulmonary hypertension in the left lung is attributed to vasoconstriction in response to increased blood flow.

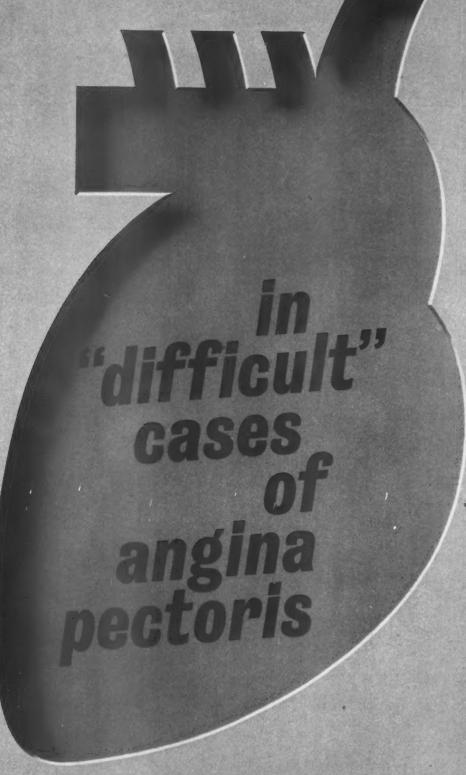
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The fortuitous-observation of primary thrombocytosis in two patients with angina pectoris suggests a relationship between these two disorders. The clinical course and electrocardiographic abnormalities of coronary artery disease in these patients seemed to vary directly with the degree of thrombocytosis. Reduction of the platelet count with radioactive phosphorus relieved the angina in both instances.

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An interesting report of a rare instance of bacteria endocarditis and rupture of an aneurysm of a sinus of Valsalva into the pericardium.



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References: 1. W. Hollander and R. W. Wilkins, in J. H. Moyer, Ed., Hypertension, Philadelphia, W. B. Saunders Co., 1959, p. 399. 2. R. W. Oblath, paper read at American Therapeutic Society, 60th Annual Meeting, Atlantic City, N. J., June 6, 1959. 3. N. Bloom, Virginia M. Month., 87:23, 1960. 4. T. Winsor and P. Zarco, Anglology, 11: (Part 2), 67, 1960. 5. G. C. Griffith, Clin. Med., 6:1555, 1959. 6. G. C. Griffith, Dis. Nerv. System, 21 (Suppl.), 101, 1960.

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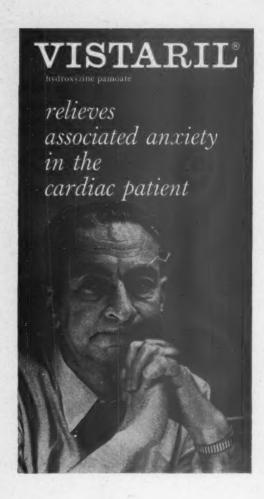
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References: 1. Russek, H. I.: Postgrad. Med. 19:562 (June) 1956. 2. Russek, H. I.: Presented at the Symposium on the Management of Cardiovascular Problems of the Aged, Dade County Medical Association, Miami Beach, April 12, 1958.

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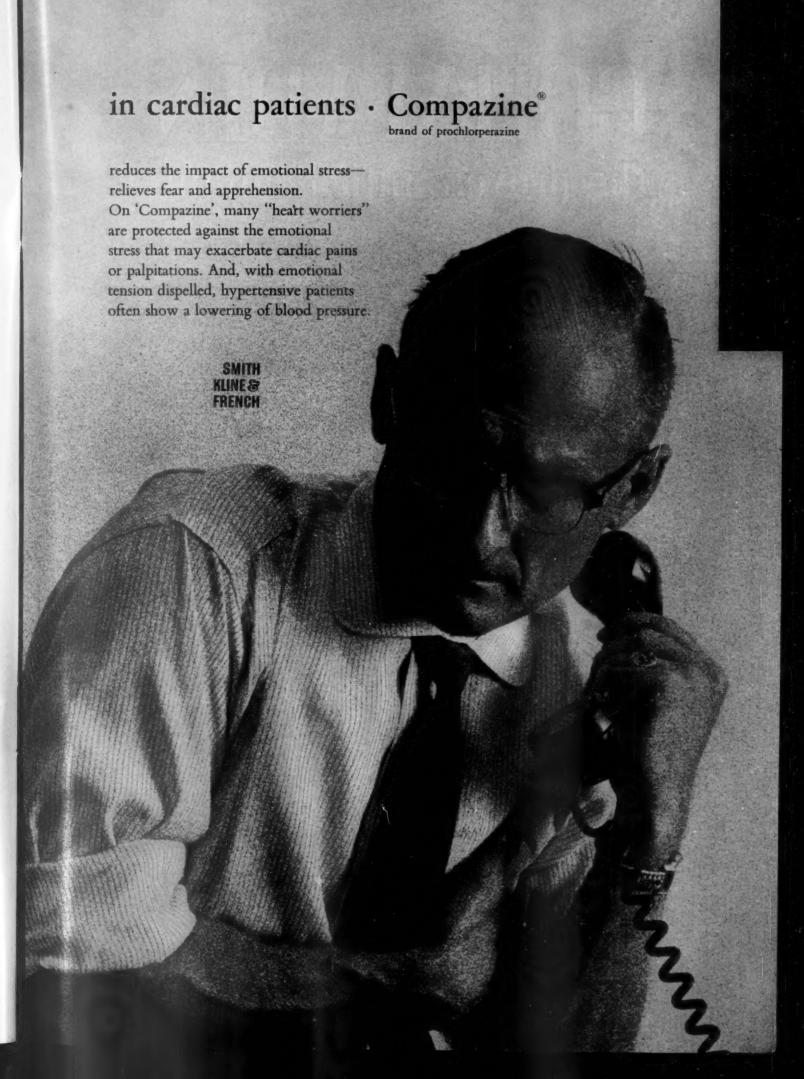
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- Feinblatt, T. M., and Ferguson, E. A.: New Eng. J. Med. 256:331 (Feb.) 1957.
 Kupersmith, I. H.: International Record of Medicine, Vol. 171 No. 10 (Oct.) 1958.
 Berry, J. W., and Roach, T. C.: Circulation, Vol. 17, No. 6 (June) 1958.

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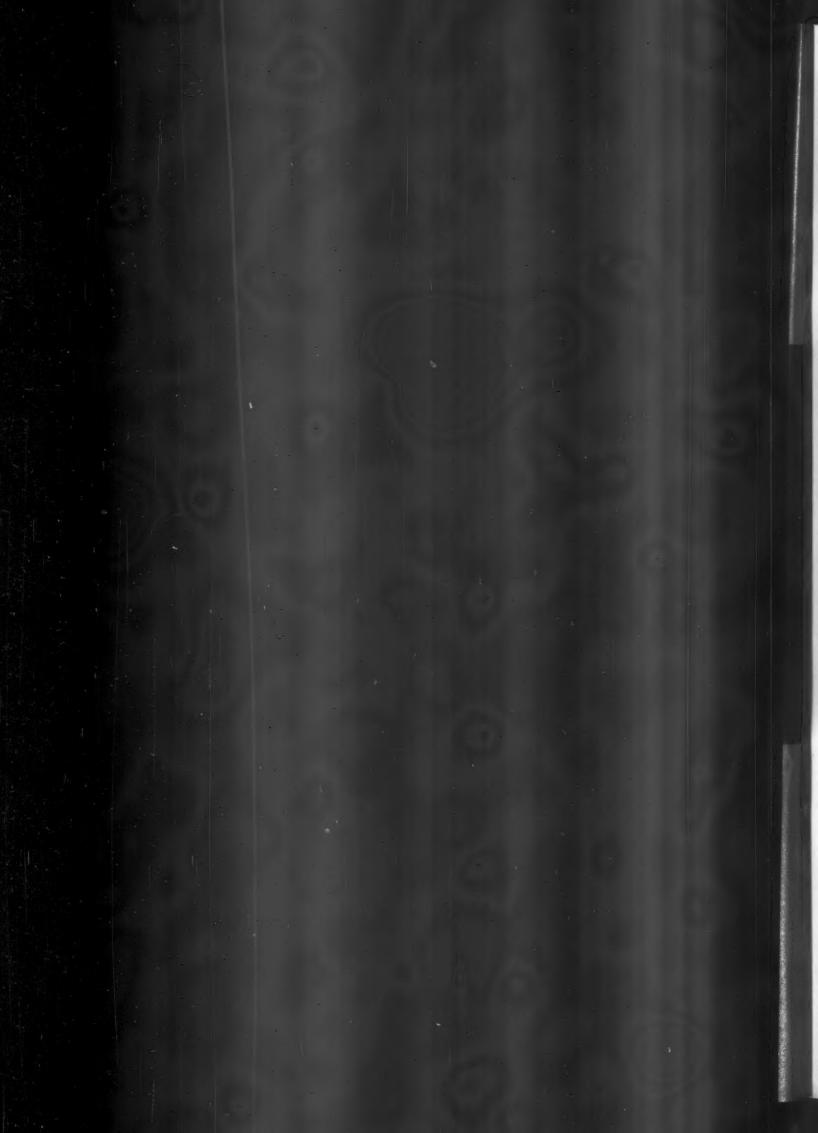
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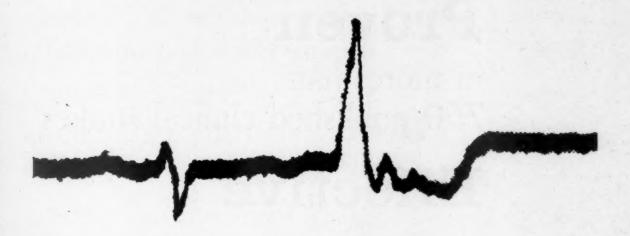
- Baer, S., et al.: J.A.M.A. 167:704, June 7, 1958.
 Moser, K. M.: Disease-a-Month, Chicago, Yr. Bk. Pub., Mar. 1960, p. 13.



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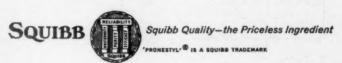
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References: 1. Zapata-Diaz, J., et al.: Am. Heart J. 43:854, 1952. 2. Modell, W.: In Drugs of Choice, C.V. Mosby Co., St. Louis, 1958, p. 454.

3. Kayden, H. J., et al.: Mod. Concepts Cardiovasc. Dis. 20:100.1951. 4. Miller, H., et al.: J.A.M.A. 146:1004, 1951.



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11. Lasagna, L.: J. Chron. Dis. 3:122, Feb. 1256. 12. Muhlfelder, W. J. et al.: Dis. Nerv. System 20:587, Dec. 1959. 13. Pollak, M.: Practitioner 184:231, Feb. 1960. 14. Rickels, K. et al.: J.A.M.A. 171:1649, Nov. 21, 1959. 15. Russek, H. L.: Am. J. Cardiol. 3:547, April 1959. 16. Tucker, K. and Wilensky, H.: Am. J. Psychiat. 113:698, Feb. 1957.

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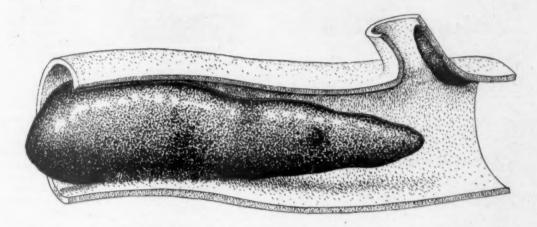
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THROMBOLYSIN, HUMAN











THROMBOLYSIN, HUMAN

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Results of therapy

Bed rest

Effect on intravascular thrombi



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Sudden death from pulmonary embolism is an ever-present hazard. One or more nonfatal pulmonary emboli may result in irreversible lung damage or secondary pneumonia.

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Weeks of hospitalization or bed rest at home are commonly required in the management of thrombophlebitis, phlebothrombosis, pulmonary embolism, and arterial thrombosis.

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Anticoagulant + Bed rest

THROMBOLYSIN + Anticoagulant + Bed rest



Anticoagulants cannot remove formed clot. However, they help prevent its extension and minimize formation of new clots.



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The careful use of anticoagulants reduces the occurrence of pulmonary emboli.



The incidence and severity of pulmonary emboli are greatly reduced since THROMBOLYSIN acts to remove thrombi before they can become emboli.



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A striking reduction is observed in the duration of hospital stay, bed rest, and convalescence.



The incidence and severity of the postphlebitic syndrome are reduced.



Postphlebitic complications are prevented or greatly minimized.











What is THROMBOLYSIN?

THROMBOLYSIN is Fibrinolysin, Human. It is prepared by activating the profibrinolysin-rich Fraction III — 3 of pooled human plasma with highly-purified streptokinase and then lyophilizing it. THROMBOLYSIN helps restore the natural equilibrium between clot formation and clot lysis, thereby enhancing the ability of the blood to maintain normal flow.

In What Conditions is it Indicated?

THROMBOLYSIN is indicated in thrombophlebitis, phlebothrombosis, pulmonary embolism, and certain arterial thrombi.

*(NOTE: Successful lysis of thrombi of major cerebral vessels has been reported. However, additional experience is required to define the indications and contraindications of therapy in such patients. THROMBOLYSIN has also been administered to patients with acute myocardial infarction, but the scope of this work is still too limited to permit conclusions about its safety or benefit.)

When Should Therapy be Initiated?

Treatment with Thrombolysin should be started as soon as possible after a thrombus has formed. Blood clots begin to organize shortly after formation and may become encased in a layer of endothelial cells, making them resistant to the action of Thrombolysin. Usually, more rapid lysis can be expected to take place when treatment is initiated within five days after a thrombus has formed; however, in some cases successful lysis has been accomplished when treatment was not initiated for several weeks after thrombus formation.

Can THROMBOLYSIN be Given to Patients

Being Treated with Anticoagulants?

Yes. Patients who have been on anticoagulant therapy can be expected to improve when Thrombolysin is added to their program of treatment.

Does THROMBOLYSIN Increase the Incidence of Embolism?

Clinical studies indicate that it does not. In fact, if any evidence of embolization should appear, it is important to continue Thrombolysin until symptoms have disappeared.

What is the Dosage?

The dosage most frequently used by investigators has been 4 vials (200,000 MSD units) per day by intravenous infusion. This is usually administered by giving 1 vial per hour for 4 consecutive hours. Alternatively, 1 vial (50,000 MSD units) per hour may be given for 2 consecutive hours and repeated in 3 to 6 hours. The dosage range is 1 vial (50,000 MSD units) to 2 vials (100,000 MSD units) an hour by intravenous drip, for 1 to 6 hours, depending on the nature of the clot and the response

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of the patient. Most patients respond in one day; those who do not may require additional doses for three or four successive days.

Patients not under active treatment with anticoagulants at the time of the thromboembolic episode:

New clot formation is unlikely to occur during the administration of Thrombolysin, so that anticoagulants may be unnecessary in this period. However, the fibrinolytic activity of Thrombolysin persists only 3 to 4 hours after cessation of infusion; in patients subject to thrombosis, provision should be made to provide adequate therapeutic anticoagulant effect at this time.

Patients under active treatment with anticoagulants:

Within recommended dosages, Thrombolysin produces only minor alterations in the clotting mechanism: the prothrombin time is generally increased by only a few seconds, the Lee-White clotting time by only 1 to 4 minutes, and the fibrinogen levels generally decrease by about 30 percent of control values. In themselves, these alterations are probably of no clinical significance. In patients on concurrent anticoagulant therapy in whom the clotting mechanism is depressed to midtherapeutic levels, the small additional depression due to Thrombolysin should produce no added danger; however, the addition of Thrombolysin may be hazardous when the therapeutic anticoagulant level already threatens to exceed safe limits.

What Other Precautions are Necessary?

THROMBOLYSIN is contraindicated in the presence of a hemorrhagic diathesis or hypofibrinogenemia. Fibrinolytic activity usually increases spontaneously for a short period after anesthesia or surgery. Therefore, THROMBOLYSIN should be used with caution because lysis of the clots at the operative site may occur.

Bleeding from open wounds or recent operative sites can occur during therapy. Usually this has been observed only in patients receiving both an anti-coagulant and Thrombolysin. In such cases, the bleeding was controlled by the use of plasma or whole blood transfusions. A specific antagonist to the anticoagulant may also be used.

What Side Effects May Occur?

Febrile reactions may occur, but these are rarely severe. When they do occur, the temperature usually rises rapidly to a peak, then returns to normal within 24 hours. In some patients, a rise in temperature above 1.5 to 2 degrees F. is accompanied by chills, nausea, vomiting, dizziness, headache, muscle pain, back pain, tachycardia, or hypotension.

How is it Supplied? 100-cc. vials containing 50,000 MSD units.

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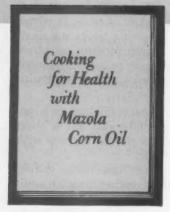








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Recommendations of leading medical authorities for dietary control of hyper-cholesterolemia include:

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- 2-limitation of total fat intake to about 1/3 of the total calories;
- 3-selection of foods so that about 1/3 of the fat intake is polyunsaturated.

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Mazola Corn Oil has the following average composition:

	Grams / 100 grams	
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Linoleates		7
Saturates, Total	11 (9-12)	1.4
Natural Sitosterols		0.14
Natural Tocopherols	about 0.1	0.015
Cholesterol	None	None
Salt (Sodium chloride) .	None	None
Calories-125/t		

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SELECTED BIBLIOGRAPHY: The methods and effectiveness of practical changes in dietary fats for hypercholesterolemic patients have been reported in numerous papers. Some recent ones are: H. B. Brown and I. H. Page. J. Am. Med. Assoc. 168, 1989-95 (1958). N. Jolliffe, S. H. Rinzler and M. Archer. Am. J. Clin. Nutrition 7, 451-62 (1959). L. W. Kinsell, G. D. Michaels, G. Walker, P. Wheeler, S. Splitter and P. Flynn. Diabetes 8, 179-88 (1959). P. A. Stefanik and M. F. Trulson. Postgrad. Med. 26, 533-8 (1959).



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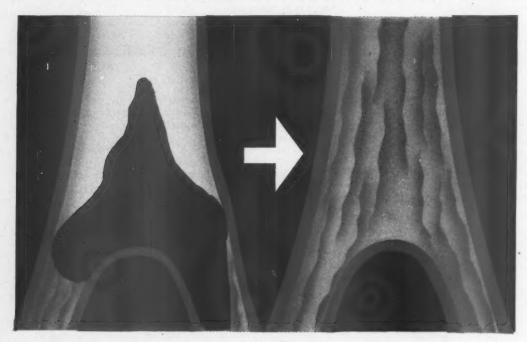
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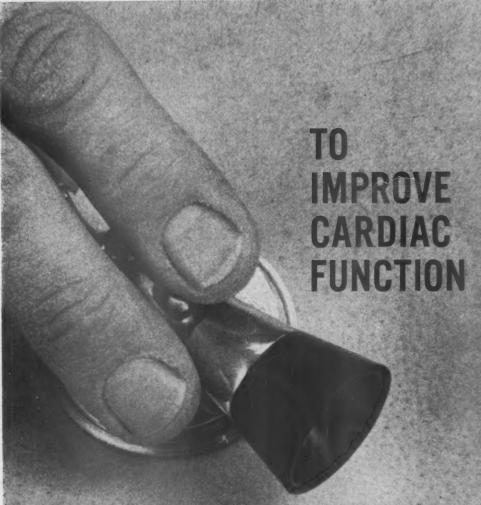
PUBLISHED CLINICAL RESULTS WITH ACTASE

Source*	Indication	Number of Patients	Results
Moser, K. M., et al.: Circulation 21:337, 1960.	Acute deep thrombophlebitis	62	decreased pain, disability, complications, reduced mortality due to pulmonary embolization — in a controlled study
Singher, H. O., and Chapple, R. V.: Clin. Med. 6:439, 1959.	Pulmonary embolism	33	70% excellent; 24% questionable; 6% poor "no untoward side effects"
Chapple, R. V., and Singher, H. O.: J.A.M.A. 173:221 (May 21) 1960.	Thrombophlebitis	171	"fibrinolysin (human) (Actase) in conjunc- tion with anticoagulant therapy decreases the morbidity in patients with thrombophlebitis."
Howden, G. D.: Canad. M.A.J. 81:382 (Sept. 1) 1959.	Central retinal vein thrombosis	1	"an excellent thrombolytic responseremarkable visual improvement"
Carroll, B. J.: Angiology 10:308, 1959.	Phlebothrombosis	82	60 excellent; 19 good; "a distinct advance in the treatment of thrombophlebitis"
Harloe, J. P.: Angiology 10:283, 1959.	Thrombophlebitis	4	"more rapid resolutionmore clear-cut clinical response"
Cliffton, E. E.: Angiology 10:244, 1959.	Peripheral venous thrombosis	38	improvement in large majority
	Pulmonary embolism	5	4 completely relieved
	Retinal vein thrombosis	2	"no further progression"
Moser, K. M.: Angiology 10:319, 1959.	Deep venous thrombosis	41	rapid response if treated within 5 days
Sheffer, A. L., and Israel, H. L.: Angiology 10:292, 1959.	Pulmonary embolism	6	4 excellent; 2 good
	Acute thrombophlebitis	9	good
	Retinal vein thrombosis	7	2 excellent; 5 no benefit
Stewart, C. F.: Angiology 10:299, 1959.	lliofemoral thrombophlebitis	2	"remarkable" in 1; "considerable improvement" in the other
Evans, J. A., and Smedal, M. I.: Angiology 10:311, 1959.	Postmastectomy thrombophlebitis of arm	10	3 asymptomatic; 5 improved; "offers promise in this field"

^{*}Reprints of these articles, as well as complete literature on intravenous fibrinolytic therapy with ACTASE, are available on request.

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The American Journal of Cardiology

VOLUME VI

AUGUST 1960

NUMBER 2

EDITORIAL

Atherosclerosis, Occlusive Vascular Disease and EDTA

AN HAS an established life span with broad individual variations which arise from inherited characteristics that shape the aging and death process and are beyond medical control. The death process begins when cellular enzyme systems become exhausted and cells can no longer divide. A person's biologic age is, therefore, related to the nature of his functional reactions when under stress. Senescence of vital organs has begun when cellular restoration to stress has been lost and healing resorts to formation of scars. The death process does vary and can cause diverse tissues to senesce or age at different times; this is true with the cardiovascular system that can then suddenly or gradually deprive blood to small or large areas of vital body structures.

It is probably true that cellular environment can be harmed by certain types of diet, prolonged emotional stress, infections and other conditions that may weaken or shorten the life of cellular enzyme reactions in some cellular structures while leaving others unchanged. Such environmental conditioners would exert their maximum effect during the earlier years of life. The mortality rate from occlusive vascular disease among young adults has been rising; if environmental factors are responsible they would have had their greatest influence during the overfed, overprotected, overindulged or emotionally frustrated childhood periods. There is reason to believe that during childhood, some undernourishment, certain chronic infections, infestations that produce prolonged secondary anemia2 and the opportunity for development of well adjusted emotional lives, with normal maturing, decrease the incidence

of premature occlusive vascular disease. When obstructive atherosclerotic plaques have developed the time has passed when we might expect new environment to change the disease process. It has been observed over the years, and with few exceptions, that the person who has had premature coronary artery disease had an emotionally unstable, poorly adjusted personality.

Diets and Lipids in Atherosclerosis: Atherosclerosis and occlusive vascular disease can be considered as synonymous; the few exceptions do not effect the over-all medical problem. Only a few years ago we accepted occlusive vascular disease as an inevitable part of growing old and therefore not amenable to treatment. The pendulum then swung to the belief that atherosclerosis was a metabolic disorder and subject to reversibility. Time has diminished this belief and, at least for some observers, has more sharply focused an understanding of atherosclerosis as a disease that is rooted in genetics but may be aggravated, especially during childhood, by various environmental conditions. Diet may play a part in occlusive vascular history but not alone or as a dominant influence. A critical attitude should be adopted when interpreting the etiologic importance of serum and tissue lipid levels. We must not base our hopes in occlusive vascular disease on experimental evidence artificially obtained, on statistics that deal with the mass without revealing innumerable variables. If we are to develop and properly appraise therapy for occlusive vascular problems we must consider each patient individually.

Disease of the arterial wall in atherosclerosis

is usually widespread and may be present for a long time but produces no symptom until it significantly occludes a vital artery. The disease is never truly segmental. Although chance does occasionally permit surgery a limited symptomatic success, particularly with abdominal arteries and occluded arteries of the leg, the disease is a major medical problem.

The present state of knowledge makes the role of lipids, and especially cholesterol, in atherosclerotic vascular disease in the adult of only academic importance insofar as it aids in preventing, controlling or relieving manifestations of the disease. The many theoretical causes of atherosclerosis have postulated infiltration of the blood plasma, invasion of arterial wall by fat-containing foam cells, lipid deposit following intimal damage, mural thrombi and primary degeneration of elastic tissue followed by incidental cholesterol deposit in areas of injury as part of the reparative process. Until a causal relation has been demonstrated between cholesterol, other lipids and occlusive vascular disease and the manifestations of the disease have been relieved by diet or lipolytic agents we must continue to think of restriction of fat in the adult diet chiefly as a means for reducing body weight.

Hemodynamic Factors in Plaque Formation: The laws of fluid dynamics help to explain the distinctive pattern of formation of atheromatous plaques in the vascular system. The early prosectors recognized this plaque distribution, located as it is at points of greatest stress and least in arteries that have freedom of expansion and retraction. While hemodynamics helps to explain the distribution of formation of arterial plaques it is an oversimplification to make this the etiology of atherosclerosis. It is true that few atherosclerotic changes appear in the pulmonary artery unless the pulmonary blood pressure is elevated and that systemic hypertension appears to increase the disease in the general vascular system. However, if hemodynamic factors caused atherosclerosis, we could expect uniform atherosclerotic arterial changes among people of the same age groups, sex and blood pressure levels. Instead we have great variation among these groups even in the presence of long standing normotension or hypertension. The forces of blood flow are counteracted by the absorptive and restorative powers of the blood vessel and the development of atherosclerosis depends on the quality and ruggedness of these resistive powers together

with the organism's capacity to restore areas of arterial injury.

Calcium Chelation and Transaminase Enzymes: In atherosclerosis a thickening of the intima due to thickened fibrocollagenous tissue and localized enlargement at sites of hemodynamic stress antedate alterations in lipids. These local internal enlargements at points of maximum stress arise from stimulated fibroblasts.3 A study of over 600 human aortas has demonstrated alterations in the medial elastic tissue and calcium content prior to the appearance of atherosclerosis.4 Changes in the medial elastic layer are responsible for subsequent formation of intimal plaques.⁵ In biologic aging of the wall of a blood vessel with loss of restorative powers the earliest manifestation of vascular wound healing is calcification of the vascular elastic membrane and localized accumulations, as well as a general increase of mucopolysaccharides.6 Heparin and other anticoagulants have had wide use in treatment of occlusive vascular disease largely because of their observed ability to diminish plasma turbidity. This action of heparin in vitro on the blood of atherosclerotic rabbits can be prevented by adding EDTA, suggesting that calcium ions are needed.

In the biologically aging artery, changes occur in certain colloids which permit easier splitting and fragmentation of elastic fibers accompanied by calcification of the fragments. The degree of calcification is directly proportional to the change in elastic tissue.7 In the healing of the vascular wound that precedes the atheromatous plaque, sulfated mucopolysaccharides play an important role and it is chiefly chondroitin sulfate that has affinity for calcium.6 It has been established that movement of cations, such as calcium, through the cell walls of tissue membranes depends upon the presence of enzyme systems. With aging an imbalance in the concentrations of enzymes within the arterial wall occurs due to loss of equilibrium between essential free ions. The cation forms a specific chelate with the protein of a specific enzyme.8 An important function of chelation in man and animals has to do with the conversion of one type of amino acid into another type or transformation of a keto acid into an amino acid, thereby restoring balances of amino acids in the body when there is the slightest lack of a vital amino acid for a particular purpose such as restoring vascular injury. The enzymes used are transaminases.9 Investigation of several transaminases has suggested that a pathologic link exists between serum enzyme activity and certain types of occlusive vascular disease.¹⁰

The vitamin pyridoxine assumes the role of an intermediate and forms chelates with various minerals to accomplish the needed conversion.9 Pyridoxine deficiency in the monkey produces atherosclerosis with hypercholesterolemia, perhaps by deficiency of specific metals in the pyridoxine-enzyme system.11 The administration of pyridoxine increases the magnesium level.12 It has been observed that in rats fed a high fat diet, atherosclerotic lesions developed but these could be prevented by greatly increasing the intake of magnesium.18 The injection of calcium into dogs with magnesium deficiency caused calcification of the heart and larger arteries that magnesium deficiency alone did not produce.14

Calcium: Magnesium Ratio: It is probable that with biologic aging, calcium in the tissues increases and magnesium decreases. This change in Ca:Mg ratio, with more ionic calcium available and shifting to soft tissues, was what suggested the use of a chelating agent in the treatment of occlusive vascular disease. A compound with in vivo ability to dissolve calcium was found¹⁵ and it was thought that it might restore essential mineral-enzyme balances. Calcium influences the colloids of cells more than any other cation. When weakened, the cell cortex of involved arterial tissues allows more calcium to enter and cause degenerative changes such as those found in atherosclerosis.¹⁶

EDTA Chelation in Treatment of Atherosclerosis: The chelating agent, EDTA, has high affinity for calcium and other minerals and was found to offer a safe mode of therapy for occlusive vascular disease. The use of this compound represented a new approach toward disease in general through control of basic and fundamental chemical processes. These chemical processes, or the enzymes that control the biochemical reactions, are sensitive to concentrations of specific metals and may be controlled by providing or withholding the required metal.

For several years we have been administering intravenously to patients with advanced occlusive vascular disease 3 to 5 gm. of the disodium salt of EDTA^{17,18} in 0.5 L. of diluent daily, a total of 90 to 150 gm. over several weeks. We realized that encouraging therapeutic results would require persistence and would vary according to the variable factors in each patient. An accumulated experience with several hun-

dred patients has demonstrated that the over-all relief from the manifestations of occlusive vascular disease has been superior to that obtained with other methods. The best results have been obtained in patients with intermittent claudication but prior to frank gangrene in whom pain at rest has been invariably relieved; all have gained materially greater ability to walk without the production of pain in the legs. There has been definite increase in the warmth and improved skin color of involved legs. In occlusive vascular disease of the brain there has been uniform relief of vertigo and tinnitus and apparent aid in recovery from acute paralysis, and the signs of senility, even when advanced, have been significantly relieved. The results in angina pectoris, while good, have not equaled those in intermittent claudication. However, there has been impressive symptomatic relief in 87 per cent of a large series of patients with angina pectoris, few recurrences of symptoms and a significant lowering of previously reported mortality rates.

The elevated serum cholesterol levels in patients who had occlusive vascular disease and in others with familial hypercholesterolemia have been greatly reduced by therapy with EDTA and in several they have remained at normal levels for as long as three years. We have used the serum cholesterol level and its changes as an aid in controlling the therapy with EDTA.

In summary, the treatment of atherosclerotic vascular complications with the chelating agent EDTA is supported by a large volume of information concerning the development of the atherosclerotic plaque, the role of calcium, the importance of the Ca:Mg ratio and perhaps of other metals in maintaining mineral-enzyme systems for the restoration or repair of vascular injuries and the demonstration of unusual symptomatic and functional improvement in patients who had advanced states of various forms of occlusive vascular disease.

NORMAN E. CLARKE, SR., M.D. Chairman, Department of Research Providence Hospital Detroit, Michigan

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Clinical Studies

Radioiodine Uptake by the Infarcted Heart*

FRITZ DREYFUSS, M.D., MOSHE BEN-PORATH, M.SC.† and JACOB MENCZEL, M.D. Jerusalem, Israel

Tagging of lesions within the body may be attempted through the use of radioisotopes, if they are selectively taken up by damaged tissues. In the case of myocardial infarcts, Yates¹ in 1952 tried to outline myocardial necroses experimentally produced in dogs by injecting P³² intravenously. He apparently succeeded in demonstrating the ensuing infarcts in the removed hearts as "radiologic holes," when preparing radioautographs from these hearts. His experiments were based on the fact that the intact myocardium would take up the radiophosphorus in contrast to the damaged heart muscle.

Stimulated by a chance observation of radioiodine uptake by a pulmonary infarct, we chose
a different approach. We had assumed that
necroses of the heart might take up radioiodine.
We were then able to report, in a preliminary
communication,² that this working hypothesis
was substantiated by clinical investigation.
Patients who, after recent myocardial infarction,
were given radioiodine orally showed significantly higher isotope counts over the precordium than over the corresponding area of
the right side of the chest.

The purpose of this paper is to outline our method and present the material studied so far.

MATERIAL AND METHODS

Material: We have investigated sixty patients with the method described herein. In several, technical difficulties discussed elsewhere in this paper, including too short a period of investigation, make evaluation of the results doubtful. These patients have been excluded from this study; forty-nine

patients are available for adequate appraisal of results. Twenty-three had suffered a myocardial infarct, fifteen mainly of the anterior wall and eight mainly of the posterior wall (Table 1). Control patients, twenty-one in number, were chosen, matching as far as possible, the series of patients with infarcts as to sex and age. Furthermore, they comprise a number of patients with heart disease other than infarction (Table 11). The patients with infarct had suffered their attack within one to twelve days prior to examination. In one patient, a twenty-day period had elapsed. The diagnosis was based on clinical, electrocardiographic and laboratory evidence, including estimations of transaminase levels in some patients, and in one control patient and in one with infarct, by postmortem examination. Five patients who presented special problems and cannot be adequately classified with either group will be discussed separately.

Method: All patients were given radioiodine in a carrier-free form in a fasting state between 8 and 9 A.M. The dose, originally 150 to 200 μ c., was soon reduced to 100 μ c., which has since been used in our patients. Experiments with a smaller dose (50 μ c.) produced insignificant results.

Isotope counts were recorded daily over the wall of the chest, beginning twenty-four hours after the ingestion of the dose of radioiodine, and extending up to two weeks whenever possible as indicated by a persistent left-to-right difference. Counts were recorded with the patient lying in bed, using a directional scintillation counter‡ at the electrocardiographic location of leads V₃ and V₅. The counter was directed as perpendicularly as possible by eyesight toward the skin at the point examined and as close as possible without exerting pressure.

Nuclear Chicago Corporation Scintillation Detector, Model DS 5-1, connected to a Nuclear Chicago Corporation 1615-B-Ratemeter.

† Present address: Texas Medical Center, Houston, Texas.

^{*} From the Department of Medicine A and the Radiation Physics Laboratory of the Radium and Tumour Institute, Rothschild-Hadassah University Hospital, and the Hebrew University-Hadassah Medical School, Jerusalem, Israel. This study was supported by a grant from the Hadassah Medical Organization Research Fund.

Table 1
Patients with Myocardial Infarction

Case No.	Age (yr.) and Sex	Diagnosis	Days Between Infarction and Administra- tion of I ¹³¹
1	66, F	Infarction of anterior wall, congestive heart failure	1
2	58, F	Infarction of anterior wall, diabetes, hyperlipemia	1
3	53, F	Infarction of anterior wall, diabetes, obesity	3 -
4	73, M	Infarction of anterior wall, diabetes	11
5	52, F	Infarction of anterior wall	20
6	70, M	Infarction of anterior wall, congestive heart failure	8
7	51, M	Infarction of anterior wall	5
8	42, M	Infarction of anterior wall	10
9	60, F	Infarction of anterior wall	9
10	53, F	Infarction of anterior wall, diabetes	5
11	56, M	Infarction of anterior wall	4
12	68, F	Infarction of anterior wall	3
13	51, F	Infarction of anterior wall	7
14	50, M	Anterolateral infarction	3
15	72, M	Infarction of anterior wall, carcinoma of colon	12
16	60, M	Infarction of posterior wall, hypertensive heart disease	2
17	60, F	Infarction of posterior wall, pericarditis	4
18	58, M	Infarction of posterior wall	3
19	48, F	Infarction of posterior wall, cholelithiasis	10
20	45, M	Infarction of posterior wall	9
21	64, F	Infarction of posterior wall	12
22	52, F	Infarction of posterior wall, diabetes	1
23	48, M	Infarction of posterior wall	5

If the technician found a spot with higher counts within the connection line of these points, for instance, in the mid-axillary line, this "hot point" would then be included in future readings. Control counts were taken at the corresponding points of the right side of the chest at the same time. Iodine uptake was estimated every day over the thyroid gland and, for determination of the body background, over both legs. Daily readings were discontinued when the figures over the precordial area became so small that they were not considerably higher than body background readings or when no left-to-right difference had been detected for about ten days. The urine of the patients was collected during the two twenty-four-hour periods following ingestion of iodine to determine excretion of the substance.

TABLE II
Control Patients

Case No.	Age (yr.) and Sex	Diagnosis
1	62, F	Cor pulmonale, congestive heart failure
	71, F	Hyperthyroidism
2 3	23, M	Duodenal ulcer
4	49, F	Essential hypertension
5	34, M	Right bundle branch block
6	32, F	Contact dermatitis
7	60, M	Bronchiectasis, old myocardial infarc- tion, congestive heart failure
8	70, M	Hypertensive heart disease, congestive heart failure
9	70, M	Hepatic cirrhosis, duodenal ulcer
10	59, M	Subacute bacterial endocarditis, ven- tricular septal defect
11	48, F	Obesity, essential hypertension
12	35, M	Polyp of rectum
13	52, F	Intraventricular conduction disturbance
14	60, F	Left hemiplegia
15	59, F	Subacute bacterial endocarditis, pul- monary infarctions, rheumatic heart disease
16	47, F	Cor pulmonale, congestive heart failure
17	60, F	Arteriosclerotic heart disease, old myo- cardial infarction, diabetes
18	59, F	Hemolytic anemia, diverticulum of stomach
19	35, M	Bilateral virus pneumonia
20	55, F	Cirrhosis of the liver
21	29, M	Perirenal abscess

The data were submitted to one of us (M. B. P.) who, like the technicians performing the counts, was unaware of the clinical diagnoses. After correcting the figures by deducting the body background counts, he established left-to-right ratios of the readings, lead V₃ left as against lead V₃ right, or hot point left as against the corresponding point on the right side, etc.* He would then make his own diagnosis as to whether the ratios obtained conformed with or disproved the diagnosis of myocardial infarction. Our criteria for this diagnosis will be discussed later.

The system of using left-to-right ratios for evaluation of the counts obtained proved superior to the consideration of absolute counts for diagnosis. Varying iodine uptake by the thyroid and other factors still unknown to us produce a great deal of variability of the absolute counts from day to day. While the daily record will frequently point clearly to the presence of an area of increased uptake, the

^{*} County_sleft - Count_{body} background (left calf)

County_sright - Count_{body} background (right calf)

L:R ratio.

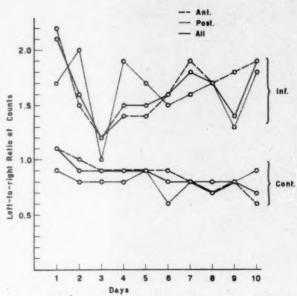


Fig. 1. Means of left-to-right ratios of radioiodine counts of twenty-three patients with infarction and twenty-one control patients.

daily recording of the left-to-right ratio proved a more reliable means of obtaining the desired information.

RESULTS

Patients with Myocardial Infarction: All patients with myocardial infarction showed an increased uptake of radioiodine over the infarcted area of the heart in comparison with the uptake determined over the corresponding area in the right side of the chest. This increased left-to-right ratio of counts was usually present one and two, and six and seven days after the administration of I131, but frequently such an elevated ratio was noticeable also on other days during the observation period. In one patient it persisted for twenty-Such consistent elevation would one days. sometimes serve as supportive evidence for the presence of an increased uptake by the heart, even when the absolute left-to-right ratios were 1.2 (borderline) or only slightly higher.

During the first few days of investigation high counts were rarely as concentrated over a specific point as they were later on. Nevertheless, topographic diagnosis (infarction of the anterior or posterior wall) was occasionally possible even then. Certainly, data obtained after six to seven days show left-to-right ratios characteristic of an accumulation of radioiodine anteriorly with the anterior localization, and posteriorly with the posterior localization of the infarct. At that time, in the infarction

of the posterior wall the left-to-right ratio over the anterior wall of the chest was frequently 1 or 0.9, whereas posteriorly it would still be 1.2 or above. Table III and Figure 1 summarize the results in all patients with infarction compared to the observations in the control patients.

Control Patients: The control patients, although exhibiting an occasional left-to-right ratio of 1.2 or more, and, on some days, overlapping the patients with infarction in their average ratios, were yet clearly discernible from the patients with infarction; they did not show a fairly constant elevation of counts over the heart as against the right side. If a hot point seemed to be present, it would soon disappear again, usually after one day. Figure 2 illustrates the left-to-right ratios in six representative patients (three with infarction and three control patients) obtained from their daily counts. Table IV summarizes the records of two patients with infarction (Cases 5 and 23, Table I) and a control patient (Case 10, Table II).

COMMENTS

The uptake of radioiodine by the infarcted heart according to the results reported apparently can be regarded as a rather constant phenomenon when the test is performed within a number of days or a few weeks following the onset of infarction. We had first expected that the absolute counts registered over the first six hours of the examination might give perhaps overlapping but distinctive ranges of figures. Our further experience has not borne this out and we have, therefore, ceased taking counts before twenty-four hours following ingestion of iodine. For a number of hours at least, counts over the heart may be expected to be higher than over the right side of the chest because of the considerable amount of I¹³¹ circulating through its chambers. This may also present a problem in patients with hypothyroidism in whom blood levels of radioactivity may be higher for at least the first two days following the oral dose. Comparative determinations of the I181 content of the blood in patients with infarction and in control patients under the conditions of our experiment have shown no significant difference between the two groups as to the rapidity with which I131 disappears from the blood; after three days counts are negligibly low in both groups. This then will not explain the difference between counts over the left as against the right areas of the chest.

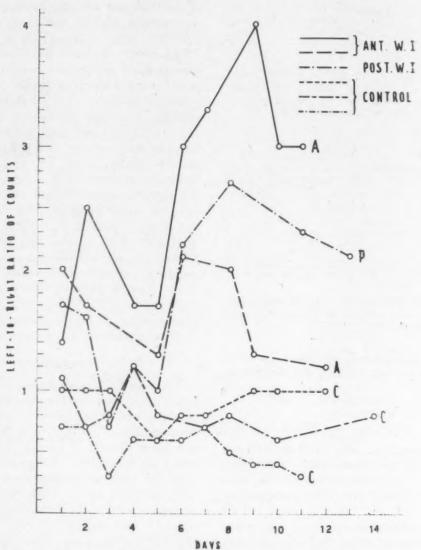


Fig. 2. Left-to-right ratios of counts in three patients with infarction and three control patients.

One patient (Case 18, Table 1) who had been given radioiodine three days after the occurrence of the infarct died on the seventh day of the investigation. His heart was examined on the eighth day; i.e., one day after his death and eleven days after the infarction. Counts obtained in tissue homogenates taken from various areas of the heart showed values which were higher in specimens taken from both infarcts than from other areas of the heart (Fig. 3). The heart of a control patient who died was also examined seven days after ingestion of radioiodine but due to the low counts at autopsy all over the heart, including the vegetations on its valves, the detected differences could not be considered significant.

It may be assumed that the damaged heart

muscle or its immediate surroundings accumulate radioiodine, but the nature of this phenomenon is still unclear. Low absolute values in some patients with low thyroid function as well as persistence of an increased uptake for twenty-one days in a patient with an infarction of the posterior wall may suggest that hormonal iodine may be responsible. On the other hand, elementary iodine may be bound to the proteins of necrotic cardiac tissue. The former hypothesis may be tested by blocking thyroid uptake, thus facilitating the clarification of any thyroid-infarct relation.

ILLUSTRATIVE CASES

Four patients observed in the course of this investigation permitted additional observations



Fig. 3. Case 18 (Table 1). Heart showing two infarctions of the posterior wall. The areas from which tissue samples have been examined for isotope content are marked. Areas ③ and ⑦ are the infarcted areas. I¹⁸¹ counts (per minute per gram): ①, 400; ②, 400; ③, 600; ④, 370; ⑥, 400; ⑥, 300; and ⑦, 600.

which in some way may contribute to the elucidation of the phenomenon described.

An eighty-two year old woman with diabetes, generalized arteriosclerosis and thrombophlebitis of the left leg served as a control patient. She showed a conspicuous difference in the counts taken over her legs six hours after the administration of I¹³¹: 6,000 per minute over the left calf and 3,800 over the right, and 1,400 and 1,000, respectively, after twenty-four hours. The counts over the thighs were: left, 7,400; right, 4,300 (six hours) and left, 2,000; right, 900 (twenty-four hours). Unfortunately, counts could not be taken the following days.

CASE 15 (Table II): A fifty-nine year old woman with subacute bacterial endocarditis showed no significant difference between left and right counts over the chest for seven days. She originally served as a control patient. On the eighth day of the examination a persistence of higher counts over the right side of the chest was noted by the technician: pulmonary infarction on the right side had occurred.

A similar course of events was observed in another patient, a fifty-six year old man (Case 18, Table 1) who, after having shown the characteristic behavior of a patient with infarct exhibited comparatively high counts over the right side of the chest after a pulmonary infarction had developed. Recently, we have had an opportunity to examine a fifty-five year old woman (Case 20, Table 11) who had biliary cirrhosis. She showed persistent high counts over the liver for several days. This

behavior may find a partial explanation in an observation by Blondheim and his associates³ who noted that radioiodine administered rectally would appear later (difference of hours) in the area of the liver in patients with cirrhosis of the liver than in control patients.

EVALUATION OF ISOTOPE COUNTS

Turning to the practical aspects of the examination here described, it should be kept in mind that any evaluation of I¹³¹ counts taken over the chest at various points has a number of pitfalls which have to be taken into account. It is of great importance that the daily counts should be carried out at exactly the same spots as possible as on previous days. We therefore mark these points on the skin. Undue closeness to the thyroid, especially during the first days, may be as misleading as approaching the stomach and the liver.

As shown in Table III and Figure 1, the separation of ranges and mean values between the two groups of patients, those with infarction and control patients, is most distinctive on the first, second, sixth, seventh and eighth days. However, since there is some overlapping, we have to emphasize the importance of using for diagnosis not only the occasional left-to-right ratios but also the day-to-day persistence of an increased ratio, if observed once, especially on the days mentioned. The dip of the curve corresponding to a decrease of the left-to-right

TABLE III

Left-to-Right Ratios of Corrected* Counts, Their Means, Ranges and the Number of Observations for Twelve Days Following I¹⁸¹ Administration in Twenty-Three Patients with Myocardial Infarction

							-	1			
				D	ays After I	181 Adminis	tration				
1	2	3	4	5	6	7	8	9	10	11	12
. ,			Means ±	Standard D	Deviation in 1	Left-to-Right	Ratios				
1								1			
2.2 ± 1.1	1.5 ± 0.5	1.3 ± 0.5	1.4 ± 0.4	1.4 ± 0.2	1.6 ± 0.6	1.0 ± 0.7	1.7 ± 0.5	1.8 ± 0.9	1.9 ± 0.8	1.9 ± 1.1	1.2 ± 0
								0.0 - 0		0.1 = 0.0	0.2
1.7 ± 0.7	2.0 ± 0.6	1.0 ± 0.3	1.9 ± 1.0	1.7 ± 0.7	1.5 ± 0.3	1.6 ± 0.2	1.7 ± 0.6	1.3 ± 0.2	1.8 ± 0.6	2.0 ± 0.3	2.0 ± 0
1.1 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	$0.9 \pm 0.$
	*			Range of	Laft-to-Righ	t Ratios					1
									1		
1.1-4.0	0.9-2.0	0.9-2.2	1.0-2.0	1.1-1.7	1.0-3.0	1.2-3.3	1.0-2.5	1.0-4.0	1.2-3.0	1.0-4.0	
0.7-1.8	0.4-1.5	0.3-1.5	0.5-1.2	0.4-1.5	0.6-1.1	0.6-1.1	0.3-1.0	0.4-1.2	0.4-0.9	0.3-1.0	0.7-1.0
									-1,		
1.2-2.9	1.5-3.1	0.7-1.3	0.8-3.5	1.0-2.5	1.2-2.2	1.4-1.8	1.0-2.7	1.1-1.4	1.1-2.6	1.8-2.3	
0.5-1.4	0.7-1.2	0.6-1.5	0.6-1.2	0.2-1.2	0.4-1.1	0.4-1.0	0.3-1.1	0.7-1.0	0.8-1.0	0.6-1.0	1.0-1.0
1.1-4.0	0.9-3.1	0.7-2.2	0.8-3.5	1.0-2.5	1.0-3.0	1.2-3.3	1.0-2.7	1.0-4.0	1.1-3.0	1.0-4.0	1.2-2.0
0.5-1.8	0.4-1.5	0.3-1.5	0.5-1.6	0.2-1.5	0.4-1.1	0.4-1.1	0.3-1.1	0.4-1.2	0.4-1.0	0.3-1.0	0.7-1.0
	*			No. o	f Examinati	ons			-		
	•										
15	14	9	11	9	10	7	7	11	3	1 5	1
31	25	27	16	23	15	18	18	15	11	6	3
			-								
7	6	5	4	7	4	4	4	3	3	2	1
15	13	13	9	13	7	10	11	10	5	3	2
22	20	14	15	16	14	11	11	14	6		2
										-	
46	38	40	25	36	22	28	29	25 .	16	9	5
	2.2 ± 1.1 1.1 ± 0.3 1.7 ± 0.7 0.9 ± 0.2 2.1 ± 1.0 1.1 ± 0.3 $1.2 - 2.9$ $0.5 - 1.4$ $1.1 - 4.0$ $0.5 - 1.8$ 15 31 7 15 22	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1.1-4.0	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	1 2 3 4 5 6 Means ± Standard Deviation in A 2.2 ± 1.1 1.5 ± 0.5 1.3 ± 0.5 1.4 ± 0.4 1.4 ± 0.2 1.6 ± 0.6 1.1 ± 0.3 1.0 ± 0.3 0.9 ± 0.3 0.9 ± 0.2 0.9 ± 0.2 0.9 ± 0.2 1.7 ± 0.7 2.0 ± 0.6 1.0 ± 0.3 1.9 ± 1.0 1.7 ± 0.7 1.5 ± 0.3 0.9 ± 0.2 0.8 ± 0.1 0.8 ± 0.3 0.8 ± 0.2 0.9 ± 0.3 0.6 ± 0.2 2.1 ± 1.0 1.6 ± 0.6 1.2 ± 0.4 1.5 ± 0.4 1.5 ± 0.5 1.6 ± 0.4 1.1 ± 0.3 0.9 ± 0.3 0.9 ± 0.3 0.9 ± 0.2 0.9 ± 0.2 0.8 ± 0.2 Range of Left-to-Righ 1.1-4.0 0.9-2.0 0.9-2.2 1.0-2.0 1.1-1.7 1.0-3.0 0.7-1.8 0.4-1.5 0.3-1.5 0.5-1.2 0.4-1.5 0.6-1.1 1.2-2.9 1.5-3.1 0.7-1.3 0.8-3.5 1.0-2.5 1.2-2.2 0.5-1.4 0.7-1.2 0.6-1.5 0.6-1.2 0.2-1.2 0.4-1.1 1.1-4.0 0.9-3.1 0.7-2.2 0.8-3.5 1.0-2.5 1.0-2.5 1.0-3.0 0.5-1.8 0.4-1.5 0.3-1.5 0.5-1.6 0.2-1.5 0.4-1.1 No. of Examinati No. of Examinati 15 14 9 11 9 10 31 25 27 16 23 15 7 6 5 5 4 7 4 4 15 13 13 13 9 13 7 12 20 14 15 16 14	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		1 2 3 4 5 6 7 8 9 Means ± Standard Deviation in Left-to-Right Ratios 2.2 ± 1.1 1.5 ± 0.5 1.3 ± 0.5 1.4 ± 0.4 1.4 ± 0.2 1.6 ± 0.6 1.0 ± 0.7 1.7 ± 0.5 1.8 ± 0.9 1.1 ± 0.3 1.0 ± 0.3 0.9 ± 0.2 0.9 ± 0.2 0.9 ± 0.2 0.8 ± 0.2 0.7 ± 0.2 0.8 ± 0.2 1.7 ± 0.7 2.0 ± 0.6 1.0 ± 0.3 1.9 ± 1.0 1.7 ± 0.7 1.5 ± 0.3 1.6 ± 0.2 1.7 ± 0.6 1.3 ± 0.2 2.1 ± 1.0 1.6 ± 0.6 1.2 ± 0.4 1.5 ± 0.4 1.5 ± 0.5 1.6 ± 0.4 1.8 ± 0.6 1.7 ± 0.6 1.4 ± 0.9 1.1 ± 0.3 0.9 ± 0.3 0.9 ± 0.2 0.9 ± 0.2 0.8	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$

^{*} Observed count minus body background reading.

ratios in several patients with infarction on the third day of observation is still unexplained.

DIAGNOSTIC IMPORTANCE OF TEST

Here then arises the question of the diagnostic importance of this test. The following case report may suitably illustrate this aspect.

CASE 15 (Table 1). A seventy-two year old man was admitted to the surgical department because of carcinoma of the colon. He reacted to a blood transfusion with a violent chill and was therefore given a large parenteral dose of antipyretic (optalgin), following which he collapsed (August 4, 1959). Pain in the chest and the sudden appearance of pulmonary edema in a patient whose previous electrocardiograms showed evidence of infarction of the posterior wall raised the suspicion that he had suffered a new coronary occlusion. Under the circumstances, neither the elevated leukocyte count

nor sedimentation rate or temperature could contribute to the diagnosis. Subsequent electrocardiograms showed multiple ventricular extrasystoles, transient S-T segment depression in leads 1, V5 and V₆, considerable decrease in size of the R wave in leads V₃ through V₅, positive T waves in leads V₅ and V₆ in contrast to previously negative T waves, and later symmetrical inversion of the T wave in leads V₃ and V₄. These changes were considered by some to be due to coronary insufficiency in an already damaged heart; by most other competent observers, however, among them those who were given no further information about the patient, as conclusive evidence of a new infarction of the anterior wall. Radioiodine was given on August 16, 1959. The patient showed a persistently high left-to-right ratio over lead V5 for several days and thus our impression that he had suffered a new infarction was confirmed. Postmortem examination on September 12, 1959, disclosed an old posterolateral

Table IV

Left-to-Right Ratios of Corrected Counts in Two Patients with Myocardial Infarction and One Control Patient

Data and I ¹³¹ Dose	Position of Counter and					Days	After	Im Ad	minist	ration				
Data and 1 Dose	Left-to-Right Ratio (L:R)	1	2	3	4	5	6	7	8	9	10	11	12	13
	VaL	4,000	3,600		2,750	2,000	2,400	2,400	3,000	1,200				
	V ₂ R		1,800		1,350									
	L:R	4.0	2.0		2.0	1.7	1.7	1.7	2.5	3.0				
lase 5 (Table 1); patient with infarction of	VaL	2,600	2,100		2,200	1,200	1,600	1,000	1,500	450				
anterior wall; 100 µc. I181 given 20 days after	V ₈ R	2,700	1,750		1,800									
infarction	L:R	0.9	1.2		1.2	1.0	1.0	0.8	1.0	0.6			***	
	PL	2,000	2,300		1,750	1,500	1,100	1,100	1,600	750				
	PR		1,900		1,300									
	L:R	1.2	1.2		1.3	1.0	0.8	0.9	1.1	1.0				
	VaL	4,000		1	1	4 000	5 200	E 000	2 500	2 010	2,000		1,800	1 00
	V ₂ R	4,100									1,800		1,750	
	L:R	1.0					1.1						1.0	
Case 23 (Table 1); patient with infarction of	VaL	3,500				4,000	5,000	4,550	2,400	1.800	1,900		1,000	1.00
posterior wall; 100 µc. Ill given 5 days	VaR	3,500				3,600	4,500	4,200	2,700	1,900	2,000		1,100	1,00
after infarction	L:R	1.0				1.1	1.1	1.1	0.9	1.0	1.0		1.0	1.0
	PL	3,100									2,100		1,800	
	PR	2,600									1,900			1,40
	L:R	1.2				1.4	1.3	1.6	1.0	1.1	1.1		2.0	1.8
	VaL	2.500	2,500	1,250	2,250		2,100		1,900	1,200	900	1,000		
	V ₃ R				3,500		3,700				2,500	3,000		
	L:R	0.7	0.7	0.3	0.6		0.6		0.5	0.4	0.4	0.3	***	
10 (T-11 -) . A-1-1 - 1-1	V ₈ L				2,900		2,400				1,600			
Case 10 (Table 11); control patient; 100 μc.	V ₄ R				2,700		4,300				3,400			
I see given	L:R	0.9	0.6	1.2	1.1		0.6		0.5	0.5	0.5	0.5		
	PL				2,200		1,350				1,150			***
	PR				4,300		2,250				1,350			
	L:R	1.4	0.9	0.9	0.5		0.5		1.1	0.7	0.9	1.0		

Note: PL = posterior left; PR = posterior right.

infarct and a more recent one, several weeks old in the pathologist's estimation, located mainly in the septum.

Using the criterion set forth previously, this procedure seems to demonstrate cardiac infarction in a high proportion of cases. Of the other methods in use, electrocardiography and more recently the glutamic oxalacetic transaminase determination must doubtlessly be regarded as the most reliable diagnostic means which come closest to the aim of actually determining the presence of tissue necrosis, although both accomplish this by an entirely different approach. The method described here represents a semidirect approach. It is an attempt to imbue the necrosis or its surroundings with a radioactive material discernible from outside. We intend to test this aspect by setting up appropriate experiments on animals with induced coronary occlusion. It is an obvious drawback that other infarcts, like some of those occurring in

the lung, may attract I¹⁸¹, but this may, under suitable clinical supervision and with the necessary critical attitude, occasionally be advantageous.

ADDITIONAL CASE REPORTS

A few patients not included in our tables for reasons evident from the following descriptions deserve mention.

Case 1. S. L., a fifty year old grocer, had been admitted in May 1956 because of an attack of sinus tachycardia accompanied by precordial pain. He then probably had suffered an infarction of the posterior myocardial wall. He was admitted again in June 1958 because of the same symptoms. At that time the high take-off of the S-T segment in leads V₂ through V₄ following a period of supraventricular tachycardia made the diagnosis of a new myocardial infarction probable. His leukocyte count was 10,300 per cu. mm., the sedimentation rate 18/39 mm. (Westergren) and the C-reactive protein in his serum was 3 plus. However, there were no Q waves in subsequent electrocardiograms. This

most probably was an instance of a rudimentary infarction of the anterior wall.

The left-to-right ratios over the anterior surface of the heart were: 1 (first day), 2.1 (second day), 0.9 (third day), 1 (fifth day), 1.9 (sixth day) and 1.1 (seventh day). This definite but only occasional rise in left-to-right ratio may have been the expression of a comparatively small infarct.

CASE 2. A sixty-seven year old woman (S. K.), following a severe hemorrhage due to ulcer, showed deep S-T segment depression in leads I, II, aVL, and V₂ through V₆. These changes became less pronounced in subsequent tracings; they were typical of coronary insufficiency. Her left-to-right ratios were:

Desiries	Day									
Position	1	2	3	4	5	6	7	8		
Anterior	2.5	1.2	1.1			1.7	0.8	0.7		
Posterior	1.5	2.0	4.0		2.1	1.7	2.9	0.8		

These ratios are suggestive of infarction of the posterior wall, a complication for which there was, however, no other evidence.

CASE 3. C. S., a sixty-one year old man, was severely ill with bronchial asthma, cor pulmonale, bronchiectasis in both lungs and uremia. He had, on the second day of observation, a left-to-right ratio of 1.4 anteriorly and posteriorly and on the fifth day a ratio of 1.5 over leads V₃ and V₅. Whether radioiodine was taken up by the bronchiectasis or by the heart could not be determined. There was no evidence of myocardial infarction nor was the radioiodine test clearly suggestive of it.

CASE 4. A seventy year old woman (C. B.) was admitted because of severe paroxysmal ventricular (?) tachycardia on July 23, 1959. This tachycardia subsided the same day and a typical left bundle branch block pattern appeared in a tracing the same day, as well as in one taken the day after. There were no other signs indicative of infarction. Radioiodine was given on July 28. Her left-to-right ratios were:

Position			Day		
Position	1	2	3	5	6
Lead V ₃	2.5	1.8	1.0	1.2	0.8
Lead Vs	1.6	1.0	0.9	1.2	0.6
Mid-axillary	1.8	1.2	1.0	1.2	0.8
Postaxillary	2.0	2.0	1.2	1.0	0.7

These ratios raise the suspicion of the presence of

cardiac necrosis but in the absence of other evidence this question must remain open.

These patients, in whom neither clinical observation nor electrocardiographic study and other laboratory evidence permitted a clear diagnosis or definite confirmation of the presence or absence of an infarct, would, if properly and decisively elucidated, have been of great importance for the evaluation of our method. At present, they represent either instances of false positive or negative results of our procedure, or, on the contrary, it is our test that truly detects the presence or absence of cardiac necrosis.

A great deal of investigation will have to be carried out before the physiologic nature of this phenomenon and its behavior in the course of cardiac infarction can be properly interpreted. We are at present attempting to find out how long the positive uptake over the infarcted heart persists by examining a series of patients with this disease four to six weeks after their coronary attack.

Although the daily readings are only a matter of several minutes and the apparatus required is now available in many hospitals, further simplification of the method should be achieved. This can be attempted by two different approaches. It may turn out that readings do not have to be carried out daily and examination on the "key days" mentioned may suffice to provide the information needed by the clinician. Secondly, an instrument may be devised on the lines of an automatic scanner or a scintillation camera, as described by Anger,4 which might make the demonstration of an infarct possible in a more accomplished manner and within a shorter period of examination.

SUMMARY

An increased uptake of radioiodine by the infarcted heart has been reported by us previously. Forty-nine patients have now been investigated: twenty-three patients with recent myocardial infarction, twenty-one control patients and five patients in whom exact diagnostic classification as to the presence or absence of infarction was impossible. In all patients with infarction, an increased radioiodine uptake over the area of the heart in comparison with the corresponding area on the right side of the chest could be observed. The increased left-to-right ratio appeared after twenty-four to forty-eight hours and was also noticeable at

least on the sixth, the seventh or the eighth day after iodine administration. The 100 μ c. dose of I¹³¹ seems to be a suitable one with the instruments employed.

The phenomenon of increased radioiodine uptake by the infarcted heart, although its nature is still poorly understood, appears to have some clinical diagnostic value.

ACKNOWLEDGMENT

We wish to express our thanks to Prof. A. Hochman, Head of the Radium and Tumour Department, for his valuable advice and criticism and his aid in setting up this investigation and to Prof. J. Gross, Head of the Department of Experimental Medicine and Cancer Research, for much helpful advice. The devoted help of our technician, Miss Sarah Kornitzer, as well as that of our colleagues of the Institute of Pathology, is gratefully acknowledged.

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A Clinical Study of Triparanol (MER-29)*

PHILIP LISAN, M.D., WILBUR OAKS, M.D. and JOHN H. MOYER, M.D., F.A.C.C.

Philadelphia, Pennsylvania

This report is concerned with the metabolic effects produced by a new drug, triparanol (MER-29). The results of preliminary investigation with this compound have previously been reported^{1,2} and the observations herewith reported are a continuation of the study with this agent over a twelve- to fifteen-month period.

MATERIAL AND METHODS

Forty-five subjects with arteriosclerotic heart disease and with a serum cholesterol level of more than 250 mg. per cent were studied. Serum cholesterol determinations were carried out by the same method and by the same technician.² The control serum cholesterol level was determined by using an average of two or more determinations. Patients showing wide control variations were not included in the study. Once the baseline was established, therapy with triparanol was instituted. All subjects involved were diagnosed as having arteriosclerotic heart disease. No dietary restriction was imposed during this investigation and the patients were urged to continue the same type of diet to which they were accustomed.

The dosage of triparanol varied from 100 mg. to 1,000 mg. a day for periods ranging up to eight months. For the first month serum cholesterol determinations were obtained weekly and, thereafter, they were carried out at monthly intervals. The subjects were followed up periodically in a special atherosclerosis clinic where blood pressure, weight and clinical response were determined. At various intervals, complete blood counts and differential counts, urinalyses, blood urea nitrogen and bromsulphalein determinations were performed. Further investigation of the biochemical effect of triparanol was undertaken in the form of serum triglyceride studies, unesterified fatty acids and fat tolerance curves, according to the method of Berkowitz et al.³

RESULTS

Effects on Blood Cholesterol: The results of the cholesterol depressant effect of MER-29 are summarized in Table 1. The subjects were divided into four dosage groups. The dosages given were 100 mg., 250 mg., 500 mg and 1,000 mg. per day. The mean changes in mg. per cent from control levels are noted at one week, two weeks, four weeks and at monthly intervals thereafter. Changes from control levels were determined by the difference between the cholesterol level of each patient at the stated interval and his own control level. The number of patients is listed in each dosage period and one standard deviation is likewise shown. It is noted that the control levels of serum cholesterol were comparable in all four groups of patients.

With a dose of 100 mg. per day, the mean change in serum cholesterol did not differ significantly from control levels for the first two months. After three months, the mean drop of 28 mg. per cent was of only doubtful significance. With administration of 250 mg. per day, the change in the serum cholesterol level after two weeks was barely significant, but the change became and remained highly significant during and after the fourth week. With 500 mg. and 1,000 mg. per day there was a depression in the serum cholesterol level which became and remained highly significant during and after the first week. Thus, there is no evidence that administration of 100 mg. per day is of any value, while doses of 250 mg. per day and higher are associated with a highly significant drop in the total serum cholesterol level.

Figure 1 shows that the change in cholesterol level with the 250 mg. dose was fairly gradual, while the change with doses of 500 mg. and 1,000 mg. per day was more precipitous. Up to and including the first month, dosage was of great significance in determining the degree of change in serum cholesterol levels. From the second month on, there was no significant relation between dosage and amount of change.

^{*} From the Atherosclerosis Unit, Section of Cardiovascular Diseases, Department of Internal Medicine, Hahne-mann Medical College and Hospital, Philadelphia, Pennsylvania. This study was supported by Grant H-4565 from the National Institutes of Health.

Table 1
Statistical Analysis of MER-29: Depressant Effect on Serum Cholesterol

Daily Dose	Control Cholesterol			Change (mg. %) from	n Control Le	evel to Post-t	reatment Mo	onth		1
(mg.)	Level (mg. %)	1/4	1/2	1	2	3	4	5	6	7	8
100	325*± 29†	- 6 ±	+ 9 ±	-21 ±	-23 ±	- 28 ±					***
250	327 ±	-12 ±	-26 ±	-47 ±	-62 ±	- 72 ±	- 82 ±	-115 ±	-91 ±	-96 ±	***
500	324 ± 50	-48 ±	-52 ±	-80 ±	-73 ± 57	-105 ±	- 85 ±	- 71 ±	-92 ±	-95 ±	
1,000	321 ± .	-68 ±	-63 ± 47	-93 ±	-74 ± 34	- 79 ±	-102 ±	- 95 ±	-79 ± 37	-98 ± 22	-102 ±

Note: Figures in bold face type indicate p <0.01.

* Figures represent arithmetic mean.

† ±1 S.D.

After the first two or three months of therapy, roughly the same plateau was reached with each dosage level (Fig. 2). This level averaged 80 to 100 mg. per cent below control levels. Even after seven or eight months there was no observable tendency of the serum cholesterol to return to control levels. The initial depres-

sion was maintained as long as the subjects continued receiving the drug. Two subjects not included in this statistical analysis showed a spontaneous elevation of their cholesterol level while on therapy and these will be discussed subsequently.

Some subjects were followed up for seven to

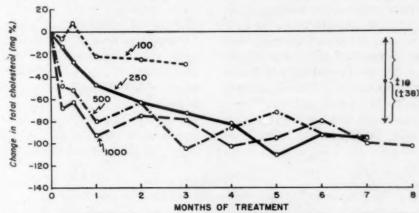


Fig. 1. Dose response curve ($1\theta = 1 \pm \text{standard deviation} = \pm 38 \text{ mg. per cent}$).

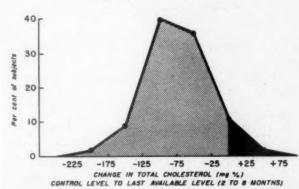


Fig. 2. Distribution curve of changes from control level to two to eight months post-treatment in forty-five subjects (dosage 250 to 1,000 mg. per day). Stippled area = cholesterol lowered significantly. Solid area = no significant lowering, or increased.

eight months and some were followed up for only two months. In each case, the first known level for each patient was recorded for doses of from 250 mg. per day to 1,000 mg. per day. Only three subjects showed an elevation in cholesterol level. In Table II it may be seen that the great majority of these drops were substantial. The serum cholesterol level was lowered significantly (more than 25 mg. per cent in 87 per cent of the subjects). It is noted that in 51 per cent of the subjects, the final serum cholesterol is more than 75 mg. per cent below the pretreatment values. Figure 2 is a distribution curve summarizing the results.

TABLE II

Distribution of Changes in Cholesterol Level*

Change in Total Cholesterol (mg. %)	No. of Subjects	Per Cent of Subjects
-225 to -176	1	2
-175 to -126	4	9
-125 to -76	18	40
-75 to -26	16	36
-25 to +24	5	11
+25 to +74	1	2

* Last available data two months to eight months after start of treatment. All dosage levels from 250 to 1,000 mg. combined.

Correlation with Fat Partition and Tolerance Studies: Fat partition studies were performed before and during therapy (Table III). This included triglycerides and unesterified fatty acids. There was no correlation between changes in cholesterol levels and the triglyceride or unesterified fatty acid levels. All of the levels for unesterified fatty acids were normal, even with elevated cholesterol or triglyceride levels.

Fat tolerance studies were carried out according to the method of Berkowitz et al.³ This consists of giving I¹³¹-tagged triolein orally and then determining the peak blood activity and the twenty-four-hour blood activity. Once again there was no correlation between changes in cholesterol and fat tolerance before or during therapy (Table III). These data seem

to indicate that the effect of triparanol in depressing cholesterol levels occurs independently of the other fat particles.

Safety Data and Toxicity Studies: Clinical side effects were found to be minimal in the dosage used. Nausea occurred in an occasional patient in whom the 500 mg. or 1,000 mg. dosage was used, but this symptom disappeared on reducing the dosage. Rashes developed in two patients. In one, the rash disappeared while the patient was still receiving the drug, and in the other, the rash disappeared when therapy was discontinued. One subject who complained of nausea also stated he had a bitter taste in his mouth. During the study, the blood pressure and weights of all subjects varied slightly. Signs or symptoms of endocrine imbalance were not observed in any patient.

Figure 3 summarizes the safety data from a laboratory aspect. Both pre- and post-treatment values were obtained for hemoglobin, hematocrit, white blood cells and blood urea nitrogen on most of the patients. There was a mean drop of 0.5 gm. per cent in hemoglobin. One of the patients had a drop from 12.2 to 8.9 gm. per cent of hemoglobin, but this was attributed to an associated diverticulitis. The remainder of the hemoglobin and hematocrit values were within normal range. There was a mean drop of 2 per cent in the hematocrit which was not significant (p > 0.10). There was a mean drop of almost 1,200 white blood cells. It is noted, however, that all the white

TABLE III

Results of Fat Partition Studies and Fat Tolerance Tests

Patient		lesterol g. %)		ycerides g. %)	Fat Tole Uni		Unesterified Fatty Acids (mEq./L.)		
	Before	During	Before	During	Before	During	Before	Durin	
F. B	400	192	95	81	Peak: 17 24 hr.: 15	12.9	0.320	0.864	
H. D.	320	300	118	204	14.0	13.7	0.612	0.606	
T. N.	337	285-240	81	197-196	11.9	13.0	0.315	0.217	
Т. Н.	420	260	74	113	11.1	17.1 8.1	0.502	0.622	
J. C.	260	162	180	186	17.7	17.7 4.0	0.276	0.324	
Normal	Less t	han 250	Less t	han 100	Maximur	m 15.0 5.0	0.4-	-0.8	

blood cell counts remained within normal limits and there were no abnormalities in the differential blood counts.

No abnormalities were noted in the urinalysis or in determinations of blood urea nitrogen. In view of the fact that almost all laboratory values remained within normal limits, these studies indicate little, if any, toxicity.

In the thirty-two bromsulphalein determinations performed, there were five abnormalities. Two of the subjects had known cirrhosis and the abnormal bromsulphalein remained at the initial level. In the other three subjects, no pretherapy level was available for comparison. It is considered that, due to a possible competitive mechanism of the drug for liver excretory function, the bromsulphalein dye may be transiently elevated. These toxicity studies are being continued in a long range program to determine whether the changes are progressive or whether the incidence of abnormality increases.

COMMENTS

Triparanol (MER-29) is an effective agent in lowering serum cholesterol. In most instances the amount of reduction in serum cholesterol was proportional to the pretherapy level. The greatest effect on the serum cholesterol level was noted in those subjects who had the highest control values.

The 100 mg. dosage was found to be of no clinical value. The 250, 500 and 1,000 mg. dosages were associated with a significant depression of the serum cholesterol level. The 500 mg. and 1,000 mg. dosages were associated with a more precipitous drop in the serum cholesterol level. With these facts in mind it is considered feasible to initiate therapy with 500 mg. or 1,000 mg. and, once the cholesterol lowering effect occurs, the dosage can be reduced to 250 mg. per day for maintenance.

The effects of triparanol in depressing cholesterol levels remain constant as long as administration of the drug is continued on a daily basis. When therapy is discontinued the serum cholesterol level will climb slowly toward control values. When therapy is reinstituted the serum cholesterol level is again reduced.

Clinical Effects of Lowered Cholesterol Levels: Clinicians are interested in the potential effect of triparanol on the prognosis of coronary artery disease. Experimental evidence, presented by Blohm, indicates that the cholesterol content of the arterial walls in animals is reduced when triparanol is used. Evidence of this in human beings, of course, is much more difficult to

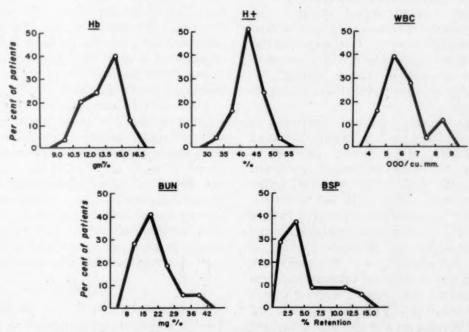


Fig. 3. Safety data: distribution curves. Hb = hemoglobin (gm. per cent), normal is 12 to 15 gm. per cent; Ht = hematocrit (per cent), normal is 35 to 50 per cent; WBC = white blood count, normal is 5,000 to 10,000 per cu. mm.; BUN = blood urea nitrogen, normal maximum is 20 mg. per cent; BSP = bromsulphalein 5 mg. per kg.; forty-five minute retention, normal: 5 per cent, equivocal 5 to 8 per cent.

elicit. However, certain clinical and laboratory findings during the fifteen-month clinical trial of triparanol show a favorable trend toward controlling atherogenesis. Conclusions can only be drawn after a five- to ten-year period of trial with triparanol.

Clinical evidence of arteriosclerotic heart disease is manifested in two ways: (1) coronary insufficiency causing angina pectoris; and (2) coronary thrombosis producing myocardial infarction. After fifteen months of therapy with triparanol two subjects suffered coronary thromboses (4 per cent) and one died (2 per cent).

Of the forty-five patients, all with coronary artery disease, sixteen had a history of frequent anginal attacks. Fourteen of the latter stated that their angina disappeared within two months after therapy was started. Several patients also volunteered the information that intermittent claudication also had improved. It is difficult to evaluate subjective symptoms such as angina pectoris and claudication since there are no reliable objective studies. Therefore, definite conclusions cannot be drawn from these results except to state that apparent improvement in coronary and peripheral arterial insufficiency is present during administration of triparanol.

Electrocardiographic studies generally showed no significant changes during therapy. In one patient, however, with a persistent pattern of coronary insufficiency (S-T segment depressions in multiple leads), there was a complete reversion to a normal tracing during triparanol therapy with associated clinical improve-

ment in angina.

In the two patients who had recurrence of their coronary thrombosis a curious situation occurred. Both had a marked response to triparanol in that their cholesterol levels fell from abnormally high values to levels below 250 mg. per cent. Both continued to receive the drug, but apparently each had a sudden elevation of the cholesterol level above normal followed promptly by another episode of coronary thrombosis (Fig. 4). The one death in this series was sudden and since autopsy was not performed we may merely surmise that it was cardiac in origin. The latter patient had an excellent response to therapy with triparanol in that his cholesterol levels had fallen to values below 200 mg. per cent and remained there until death.

Mechanism of Cholesterol Lowering: Recent

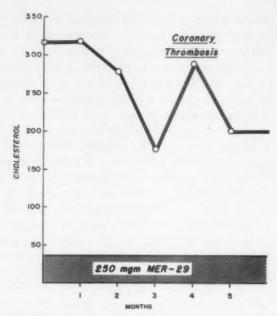


Fig. 4. Coronary thrombosis during therapy in one patient (J. E.). Unusual response to MER-29.

work by Avigan and Steinberg⁵ has given further information regarding the enzyme block produced by triparanol and the accumulation of cholesterol precursors in the blood. It is now fairly well established that the sterol which accumulates is 24-dehydrocholesterol. The atherogenicity of this new compound now remains to be determined in an effort to further clarify the value of triparanol. Previous work by Blohm and his associates⁴ indicates that the block in biosynthesis of cholesterol is at the squalene level. The studies previously mentioned⁵ indicate that the block occurs even later in the cycle.

There is also some question concerning the relation of this new sterol to the colorimetric determinations for serum cholesterol. It has been shown that following triparanol therapy, about 50 to 60 per cent of the total sterol in the blood is 24-dehydrocholesterol. This compound develops a color reaction less intense than cholesterol but which still interferes with the eventual result. In view of this work it may be necessary to re-evaluate the methods used for serum cholesterol determination.

SUMMARY

Triparanol (MER-29) is a compound which inhibits the synthesis of cholesterol in the liver. The site of action of triparanol is considered to be at the level of conversion of 24-dehydrocholesterol to cholesterol.

The following results were obtained in forty-

five patients with hypercholesterolemia: The study was performed over a twelve-month period and a dosage range of 250 to 1,000 mg. was used. With all dosages combined, 87 per cent of the patients had a significant depression in the serum cholesterol level. In 51 per cent the serum cholesterol was lowered more than 75 mg. per cent. The degree of reduction in the serum cholesterol level appears to be dependent on the pretherapy cholesterol level. The higher the initial cholesterol level, the greater the response to triparanol.

Safety data in this study revealed little or no clinical, hematological, renal or hepatic toxic effects. It is thus considered safe for clinical

It is considered that at least a five- to ten-year study is needed to determine what effect this drug will have on the prognosis of coronary artery disease.

ACKNOWLEDGMENT

Dr. Hyman Menduke performed the statistical evaluation. Dr. Robert McMaster, The Wm. S. Merrell Company, Cincinnati, Ohio, supplied MER-29.

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Prognosis of Angina Pectoris and Myocardial Infarction

Further Report

Louis H. Sigler, M.D., F.A.C.C. Brooklyn, New York

In A previous report¹ I analyzed a series of 1,700 cases of coronary disease observed over a period of twenty-five years. The series consisted of 1,302 men and 398 women. Of these, 679 patients had died and 1,021 were living at the time of that report. The present report is a follow-up study of the remaining 1,021 patients during an additional period of nine years since the last report was compiled.

RESULTS OF FOLLOW UP

This series of 1,021 consisted of 814 men and 207 women. Of these, 370 men and 86 women could not be traced or followed up, leaving a balance of 444 men and 121 women or a total of 565 patients who were followed up over this additional nine-year period. Of the 444 men, 217 have died and 227 are living. Of the 121 women, seventy-eight are dead and forty-three are alive. In other words, of the entire remaining series of 565 patients who were known to be alive nine years ago, 295 died during this nine-year period and 270 were alive at the end of this period.

Length of Survival: The length of survival from the onset of the clinical manifestations of the disease to death of those who died is shown in Table 1. In separate columns are included the combined survival periods of the 679 patients of the previous report who had died plus the 295 patients in this report, giving overall averages for the entire series of 974 patients of the original 1,700 who are known to have died.

It will be observed that the mean lengths of survival of the patients who died during this nine-year interval were 8.6 years for men and 6.9 years for women. The mean lengths of survival of the entire group who died, includ-

ing those who died during this nine-year interval plus those over the previous twenty-five years, were 6.2 years for men and 5.6 years for women. Thus, the mean longevity of the group followed up is greater than the combined longevity and the latter is greater than the longevity of the original group of those who died, which was 4.7 years for men and 4.5 years for women.

Of the 217 men who died during the nineyear interval, as many as 76 per cent were alive at the end of five years; 26.7 per cent were alive at the end of ten years; 7.4 per cent were alive at the end of fifteen years; and 3.7 per cent were living at the end of twenty years. One patient lived about forty years after the onset of clinical manifestations of the disease. In the combined group of 705 men who died, only 50 per cent were alive at the end of five years; 15.2 per cent at the end of ten years; 4.7 per cent at the end of fifteen years; and 1.7 per cent at the end of twenty years. Of the seventy-eight women patients who ultimately died during the nine-year period, 55.1 per cent were alive at the end of five years; 14.1 per cent at the end of ten years; 3.8 per cent at the end of fifteen years; and 1.3 per cent at the end of twenty-five years (one patient who died twenty-six years after the onset of the disease). These findings are higher than those of the original group of 488 men and 191 women who had died before this nine-year interval. In that group, only 32.8 per cent of the men and 34.5 per cent of the women were alive at the end of five years; 10 per cent of the men and 9.4 per cent of the women were alive at the end of ten years; 3.5 per cent of the men and 3.7 per cent of the women at the end of fifteen years; and only 0.4 per cent of the men at the end of twenty years.

Table 1

Length of Survival of 295 Dead Patients with Coronary Disease in this Series and Combined with
679 of the Previous Series

			N	Men .					We	omen		
Years After	P	resent Se (217)	eries		Combined with Previous Series (705)			resent Se (78)	eries	Combined with Previous Series (269)		
Onset	No.	Still	Living	No.	Still	Living	No.	Still	Living	No.	Still Living	
	Dead	No.	%	Dead	No.	%	Dead	No.	%	Dead	No.	%
1/2				28	677	96.0				12	257	95.
1	7	210	96.8	54	623	88.4	2	76	97.4	15	242	90.0
2	10	200	92.2	68	555	78.7	5	71	90.8	29	213	79.
3	10	190	87.6	84	471	66.8	10	61	78.2	44	169	62.
4	12 .	178	80.0	70	401	56.9	9	52	66.7	32	137	50.
5	13	165	76.0	76	325	50.5	8	44	55.1	27	110	40.
6	28	137	63.1	-70	255	36.3	8	36	46.2	22	88	32.
7	20	117	53.9	50	205	28.9	6	30	38.5	17	71	26.
8	19	98	45.3	35	170	24.0	8	22	28.2	-16	55	20.
9	19	79	36.4	29	141	20.0	3	19	24.4	11	44	16.
10	21	58	26.7	34	107	15.2	8	11	14.1	15	29	10.
11	17	41	18.9	28	79	11.2	1	10	12.8	3	26	9.
12	11	30	13.8	17	62	8.8	4	6	7.7	12	14	5.:
13	6	24	10.1	11	51	7.2				1	13	4.
14	4	. 20	9.1	10	41	5.8	2	4	5.1	2	11	4.
15	4	16	7.4	8	33	4.7	1	3	3.8	5	6	2.
16	2	14	6.5	5	28	4.0	i	2	2.6	2	4	1.
17	5	9	4.1	7	21	3.0				1	3	1.
18				2	19	2.7				i	2	0.
19				1	18	2.6	1	1	1.3	1	1	0.
20	1	8	3.7	6	12	1.7						1
21	3	5	2.3	5	7	1.0	***	***	***	***		
22	2	3	1.4	2	5	0.7			***			
23	1	2	0.9	1	4	0.6						* * *
25				1	3	0.5						***
26	***			-			1	0	0.0	1	0	0.0
27	4		0.5	1	2	0.3				1		0.0
30	1	1.	0.5	1 1		0.3		***	***			
35			17.7.4	1	1	0.1						
				1	1	0.1	3,11		* * *		***	
40	***		***	1	0	0.0			* * *		* * *	
Mean		8.6 yr.			6.2 yr.			6.9 yr.			5.6 yr.	

The length of survival of 227 men and forty-three women who were alive at the end of this nine-year interval is shown in Table II. The average mean survival for men was as high as 11.5 years and for women eleven years. The greatest percentage of men was alive between ten and fifteen years after onset of the disease. The greatest percentage of women was alive between nine and thirteen years after onset.

Number and Percentage of Patients Alive in the "Living Group" at Given Years: Table III

shows a few representative years, at the end of which the number and percentage of patients were still alive in the living group of 227 men and forty-three women. It is noted that as many as 91.2 per cent of the men and 88.3 per cent of the women were alive five years after the onset of the disease; 65.2 per cent of the men and 58.1 per cent of the women ten years after the onset; and 11 per cent of the men and 16.3 per cent of the women, fifteen years after the onset. There was thus a slightly

Table II Length of Survival of 270 Living Patients with Coronary Disease

Years After		Men (227)		omen 43)
Onset	No.	%	No.	%
1	4	1.6	1	2.3
2	5	2.2	1	2.3
	6	2.6	1	2.3
4	1	0.4		
5	4	1.6	2 2	4.7
6	9	4.0	2	4.7
7	10	4.4		
8	8	3.5	3	7.0
9	7	3.1	3	7.0
10	25	11.0	5	11.6
11	31	13.7	7	16.4
12	31	13.7	3 5 7 5 5	11.6
13	20	8.8	5	11.6
14	29	12.8	1	2.3
15	12	5.3		
16	4	1.6	3	7.0
17	6	2.6		
18	4	1.6	1	2.3
19	1	0.4	1	2.3
20	2	0.9	1	2.3
21	1	0.4		
22	2	0.9		
23	1	0.4	1	2.3
24	1 1	0.4		
27		0.4		
29	1	0.4		
30	1	0.4		
Mean	11	5 yr.	11	yr.

greater percentage of women than men who were alive fifteen years after the onset of the disease.

Age at Death: Table IV shows the age at death in decades of the 217 men and the seventy-eight women who died during this interval of nine years. Accompanying these figures

are the combined figures and percentages of this group plus the 488 men and 191 women who died before this nine-year interval who were reported on previously. It will be observed that the mean age at death in men of this series was 61.3 years and in women, 66.9 years. In the previous report of 679 deaths, the mean age for men was 58.8 years and for women, 61.7 years. In the combined groups the mean for men is 60.1 years and for women, 64.3 years. The youngest and oldest ages for males in this series are thirty-seven years and eighty-three years for men, and for women, twenty-six years and eighty-five years, respectively. In the previous series the youngest and oldest ages for men were twenty-three and eighty-five years and for women twenty-five and ninety-four years, respectively. The greatest percentage of deaths for both men and women occurred between fifty and seventy-nine years. However, women showed a relatively greater percentage in the older age group than men.

Number and Percentage of Patients Still Alive at Given Ages: In patients who were still alive when this report was compiled (Table v), the mean age for men is 60.8 years and for women, 61.9 years. The youngest and oldest ages for men were thirty-six and eighty-four years, and for women, forty-two and eighty-six years, respectively. The combined largest percentage of living for both series was between fifty and seventy-nine years with men being inclined to the lower ages.

Duration Between First Attack of Coronary Occlusion and Present Age of Living Patients: Of the 444 men and 121 women in this series there were 371 men and 67 women who had experienced one or more attacks of coronary occlusion with myocardial infarction. Of these, 193 men and twenty-seven women were still living and 178 men and forty women died

Table III

Number and Percentage of Patients Alive During the Given Years in the Living Group of 227 Men and Forty-Three Women

Sex		At End of 4 Yr.		At End of 5 Yr.		End of Yr.	At End of 15 Yr.		
	No.	%	No.	%	No.	%	No.	%	
Males Females	211 40	93.0 93.0	207 38	91.2 88.3	148 25	65.2 58.1	25 7	11.0	

TABLE IV

Age at Death (in Decades) of 217 Men and Seventy-Eight Women of this Series and Combined with the 488 Men and 191 Women of the Previous Report

			Men		Women			
Ages (yr.)	Present Series (217)		Combined Series (705)		Present Series (78)		Combined Series (269)	
	No.	%	No.	%	No.	%	No.	%
20-29			2	0.02	1	1.3	3	1.1
30-39	1	0.04	12	1.7			3 3	1.1
40-49	18	8.3	74	10.5	2	2.6	21	7.8
50-59	68	31.3	239	33.9	18	23.1	73	27.2
60-69	85	39.2	260	36.9	32	41.0	110	40.9
70-79	39	18.0	101	14.3	19	24.4	46	17.1
80-89	6	2.7	17	2.4	6	7.8	11	4.1
90 and over		• • •				***	2	0.7
Mean age		51.3	6	0.1	6	6.9	6	4.3
Youngest	1	37	3	0	2	6	2	5.5
Oldest	. 8	33	8	4	8	5	8	9.5

during this nine-year interval. Table vi shows the number and percentage of patients still living at given years after the first attack of coronary occlusion. The mean number of years was 10.9 for men and 10.8 for women. As many as 124 (64.1 per cent) of the men and fifteen (55.5 per cent) of the women are alive ten to fourteen years after the first attack of occlusion. The number diminishes with the increase in the number of years. It is also smaller in all the years up to ten years after the attack.

TABLE V
Present Age of Living Patients

Ages (yr.)	_	Men 227)	Women	
(91.)	No.	%	No.	%
30–39	1	0.4		
40-49	19	8.4	1	2.3
50-59	76	33.5	9	20.9
60-69	96	42.3	26	60.5
70-79	32	14.1	6	14.0
80–89	3	1.3	1	2.3
Mean Age	60.8		1	1.9
Youngest Oldest	36 84		8	

Tanta

Duration Between First Attack of Coronary Occlusion and Present Age of Living Patients

Time (yr.)		Men (93)	Women		
(91.)	No.	%	No.	%	
1	5	2.6	1	3.7	
2	5	2.6			
2 3 4	6	3.1			
4	1	0.5			
5	2	1.0			
6	7	3.6	2	7.4	
7	8	4.1			
8	6	3.1	1	3.7	
9	6	3.1	5	18.5	
10	24	12.4	4	14.8	
11	30	15.5	4	14.8	
12	29	15.0	3	11.1	
13	18	9.3	4	14.8	
14	23	11.9			
15	9	4.7	1	3.7	
16	3	1.6	1	3.7	
17	4	2.1			
18	3 2	1.6			
19	2	1.0			
20		0.5			
22	1	0.5			
23			1	3.7	
Mean	10	0.9	1	0.8	

TABLE VII

Duration Between First Attack of Coronary Occlusion and Death

		M	len .			Wo	men	
Time (yr.)	Present Series (178)		Combined Series (474)		Present Series (40)		Combined Serie	
	No.	%	No.	%	No.	%	No.	%
Less than 1 mo.			21	4.4			11	6.2
1-2 mo.			14	3.1			11	6.2
3-6 mo.			28	5.9			16	9.2
7 mo1 yr.	7	3.9	48	10.1	1	2.5	19	11.2
2	7	3.9	41	8.6	5	12.5	24	14.1
3	7	3.9	45	9.5	7	17.5	22	13.0
4	10	5.6	37	7.9	7	17.5	25	14.6
5	10	5.6	37	7.9	3	7.5	6	3.4
6	26	14.6	45	9.5	5	12.5	5	2.8
. 7	16	9.0	23	4.9	4	10.0	6	3.4
8	17	9.6	25	5.2	7	17.5	- 10	5.7
9	21	11.8	27	5.7	2	5.0	4	2.1
10	17	9.6	21	4.4	3	7.5	7	3.5
11	15	8.4	22	4.6	1	2.5	2	1.0
12	9	5.1	15	3.2	2	5.0	3	1.5
13	6	3.4	10	2.1			1	0.5
14	2	1.1	2	0.4	1	2.5	1	0.5
15	1	0.6	2	0.4			2	1.0
16	1	0.6	2 2	0.4				
17	3	1.7	4	0.8				
18			1	0.2	***			
19					1	2.5	, 1	0.5
21	1	0.6	1	0.2				
22	1	0.6	1	0.2				
26					1	2.5	1	0.5
27	1	- 0.6	1	0.2				
30		***	1	0.2			***	
Mean		8	5	. 4	Q	. 3	4	.2

Duration Between the First Attack of Coronary Occlusion and Death: Table VII shows the number and percentage of patients who died in given years in the present series and in the combined group of this series plus that reported previously. It will be noted that the mean number of years the patients lived after the first attack of myocardial infarction in the present series was eight for men and 8.3 for women. In the combined series it was 5.4 for men and 4.2 for women. A relatively greater percentage of men in this series lived eight to thirteen years after the infarction and a relatively greater percentage less than six years in the combined male series. Approximately the same is true of women. There was a tendency for women to have survived a shorter number of years than men.

COMMENTS

It is evident from our follow-up study of 1,700 cases of angina pectoris and myocardial infarction over a period of thirty-four years that the prognosis is far better than has been considered heretofore by others. Most reports in the literature quoted in my previous paper consisted of small series of patients observed over a relatively short period of time and do not depict the true prognosis. I could find no subsequent reports to modify this statement. The longest previous period of observation of a series of patients was that of White and coworkers.2 They reported on 500 patients whom they observed over a period of twentythree years. At the end of that time 445 had died and fifty-five were living. The average duration of life for those who had died was seven

years, and for the living eighteen years. Gubner and Ungerleider,³ apparently from a study of life insurance statistics, state that the mortality in the first two years after a coronary attack is six to seven times the normal death rate and it falls progressively thereafter with increasing length of time after the attack.

The first part of my report published in 1951 had some fault insofar as new cases have been added continuously over the period of twenty-five years. This fault has been minimized by careful history taking as to the exact time of the onset of the clinical manifestations of the disease in each case. The follow-up study of the 1,021 patients who were alive nine years ago, over the subsequent period of nine years has the advantage that no new patients have been added during this period. The report covers only the remaining 1,021 patients of the original series of 1,700. We must bear in mind the fact, however, that 456 patients have been lost track of during this period of nine years, which would probably modify our findings somewhat if we were able to see them. However, even if we assume that these 456 patients died during this period, the length of survival and other features in the statistical study would probably not differ from the group whose death was known.

It is evident from our findings that the exact longevity cannot be determined, even from a large series of patients observed over a long period of time, until the last patient of the series has died. Thus, although the average length of survival of the 217 men and seventy-eight women who died was 8.6 and 6.9 years, respectively, over the last nine-year period of observation, the actual average survival for those in the entire series of 1,700 who had died was only 6.2 and 5.6 years for men and women, respectively. Inasmuch as there were still 270 known living patients, the ultimate average longevity will be much higher for the entire series.

It is of interest to find that of the 270 patients who were still living at the end of the thirty-four years of observation the average longevity was already much higher than that of the group who had died, being 11.5 years for men and eleven years for women. This average is lower than that of eighteen years for the fifty-two remaining living patients reported on by White and co-workers at the end of twenty-three years. The number of living patients there, however, was relatively small. Also, as

shown in Table III, of our 227 men and forty-three women alive at the end of the thirty-four-year period, as many as 148 men and twenty-five women were living at the end of ten years of follow up and twenty-five men and seven women at the end of fifteen years. If we follow up this group of 227 men and forty-three women several years longer the average longevity of the remaining living will probably be much greater.

The excellent prognosis of many patients suffering from the disease is shown by a comparison of their longevity with the average life expectancy. In 1957, the average life expectancy was 67.1 years for men and 73.5 years for women. In our series of the remaining 565 patients followed up over a period of thirty-four years, 27.6 per cent of the men and 15.4 per cent of the women who died had lived beyond this life expectancy. Of those who were alive at the end of this period of study, 23.4 per cent of the men and 7 per cent of the women have already surpassed that expectancy.

Like the longevity, the mean age at death is greater for both men and women in the group followed up for the past nine years than in the previous years, and a greater percentage of patients lived beyond sixty years of age. It is also of interest to find that the longevity of those patients who had had at least one attack of myocardial infarction was not much shorter than of the entire series, which includes many who had experienced no such attack.

In the ultimate prognosis of coronary disease we must take into consideration the cause of death of those patients who died. In my previous report I found that 83.5 per cent died from heart disease and 16.5 per cent from other causes. These figures remain about the same for this remaining group of patients. In the series reported by White and co-workers only 76 per cent of deaths were due to heart disease.

CLINICAL SIGNIFICANCE OF DATA

Etiologic Factors: The marked differences in the longevity of patients with coronary disease and the generally favorable prognosis, also the frequent remissions of acute manifestations of the disease with freedom from symptoms in many for months or years speak for intermittency and variability of the causative factors of the disease.

It is not the purpose of this paper to discuss the possible causative factors. I will merely stress the fact that the underlying causes of the disease appear to be multiple and their effects have not been sufficiently studied in the past. Our main attention is being concentrated on laboratory studies of lipid and cholesterol metabolism as the chief or only underlying causes of the disease. Although these studies are highly illuminating and appear to show a link between such disturbances and the pathologic lesions in coronary atherosclerosis, the main etiologic problems are far more complex. Thus, recent studies appear to point to the fact that even disturbances in lipid and cholesterol metabolism and changes in the coagulability of blood may to a great extent be induced by emotional strain.5-8 Future studies may reveal that many of the hemodynamic, hematologic, and physicochemical changes responsible for the disease are induced by a variety of conditions and will help solve the problem of the pathogenesis of the disease.

It may be of help in the future evaluation of long term anticoagulant therapy to know that none of the 1,700 patients reported on in this study received such treatment.

SUMMARY

This is a follow-up report of 1,021 patients with coronary disease studied over an additional period of nine years. The original study of 1,700 cases1 covered a twenty-five-year period. Five hundred sixty-five of these patients were traceable or were under observation during this additional nine-year period. Of these, 217 men and seventy-eight women had died and 227 men and forty-three women were alive at the end of this nine-year period. The average length of survival of those who had died was 8.6 years and 6.9 years and of the living group 11.5 years and eleven years for men and women, respectively. In the living group as many as 91.2 per cent of the men and 88.3 per cent of the women were alive at the end of five years; 65.2 per cent and 58.1 per cent at the

end of ten years; and 11 per cent and 16.3 per cent at the end of fifteen years. The mean age at death was 61.3 and 66.9 years for men and women, respectively. The greatest percentage of deaths was between fifty and seventynine years of age. The mean ages of the living group were 60.8 and 61.9 years for men and women, respectively. The mean number of years of the living patients after the first attack of coronary occlusion were 10.9 and 10.8 for men and women, respectively. In the group that had died the mean number of years the patients lived were 8 and 8.3 for men and women, respectively.

Based on our findings, the prognosis of this disease is far better than that reported in the literature heretofore. From 7 to 27.6 per cent of the patients surpassed the average normal life expectancy, and many are still living.

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Long Term Influence of the Beck Operation for Coronary Heart Disease*

BERNARD L. BROFMAN, M.D., F.A.C.C.

Cleveland, Ohio

In the past ten years, more than 600 patients with coronary heart disease have been operated on by Dr. Claude S. Beck in Cleveland; 400 were operated on since January 1954. 1-8 The over-all "early" (operative and hospital) mortality is approximately 5 per cent. Of all patients surviving operation in the past six years, the "late" mortality (up to six years after operation; average, three years) is less than 15 per cent. At least 90 per cent of the patients still living have achieved and maintained a highly satisfactory result.

In its present form the Beck operation for coronary heart disease has evolved from the so-called "Beck II operation," and from the various modifications of the "Beck I operation." ⁴⁻²¹ Since 1954, a more or less standardized surgical technic has been applied with no significant change in the criteria or selection of patients for operation.

This report is a long term follow up of a consecutive series of 110 patients discharged from the hospital in the two-year period, 1954 and 1955. In every case, I have carried out the preoperative evaluation and, as of December 1958, I have accounted for every patient operated on. The shortest follow-up period is three years, the longest five years, with an average follow-up period of four years.

At least 100 other patients with coronary heart disease were considered acceptable for operation during this two-year period but were not operated on for various reasons. This group may eventually provide an adequate control series.

PREOPERATIVE EVALUATION AND CLASSIFICATION

Angina Pectoris: Of the various symptoms associated with coronary heart disease, cardiac pain (angina pectoris) or its equivalent is the

primary consideration in the evaluation of the patient. All 110 patients had some cardiac pain, varying from mild discomfort on extreme exertion to continuous pain, even at rest (status anginosus). No correlation existed between the degree of pain and the extent of underlying disease in the coronary arteries and myocardium. The most dramatic results from operation occurred in patients with a maximum of symptoms and a minimum of disease.

Classification of patients with coronary artery disease is difficult. Consideration must be given not only to the degree of myocardial degeneration but also to the progression of the occlusive process in the arteries. The following preoperative classification is useful: (1) Mild: angina occurs only with strenuous activity and is readily relieved by rest or by administration of nitroglycerin. Average dosage is one nitroglycerin tablet per day. These patients may have had a small infarction previously but are able to work full time. (2) Moderately severe: angina regularly occurs with mild to moderate degrees of effort. The patient uses two to eight nitroglycerin tablets per day with good results. He may have some myocardial scarring. He is able to work more than half the time. (3) Severe: angina occurs even at rest (status anginosus). Administration of nitroglycerin may not relieve symptoms. There may be rapid progression and extensive scarring present. The patient is unable to work full time and his economic productivity is significantly reduced.

The preoperative classification of the 110 patients in this series was: (1) mild, twenty patients; (2) moderately severe, forty-five patients; and (3) severe, forty-five patients.

Age: The ages ranged from twenty-seven to sixty-seven years, with an average age of

^{*} From the Hexter Cardiopulmonary Laboratory, Mount Sinai Hospital, and University Hospitals, Cleveland, Ohio.

Table I

Age Distribution of 110 Patients Operated On for Coronary Heart Disease

Age (yr.)	Total	Alive at Present	Late Deaths
Less than 30	3	3	0
31-40	21	16	5
41-50	48	34	14
51-60	34	31	3
61-70	4	2	2
Total	110	86	24

forty-seven years (Table 1). Generally, patients over sixty-five years of age have a somewhat higher operative risk, but operation is not denied such a person if his "tissue age" justifies it. Four patients in this series were over sixty years of age. With respect to the twenty-four patients under forty, the rapidly progressive disease frequently present in this age group tends to make operation somewhat more hazardous.

Sex: Of the 110 patients who survived operation, only five were women, two of whom were less than fifty years of age (forty-four and forty-eight years). All five are still alive and, with one possible exception, have had excellent results.

Duration of Symptoms Prior to Operation: The duration of symptoms averaged two and a half years, with a range of six months to thirteen years. (In the twenty-four late deaths, the

average duration of symptoms was four years.) Thirty-three per cent had symptoms for one year or less. An equal number had symptoms for one to three years, and 20 per cent from three to five years. Fifteen per cent had symptoms for more than five years.

Previous Myocardial Infarction: Of the 110 patients, 20 per cent had no clinical episode of acute myocardial infarction. In 40 per cent there had been two or more episodes of acute myocardial infarction; there was an average of 1.5 episodes of myocardial infarction per patient. If performed early, operation may well reduce the mortality rate of 20 per cent associated with the first attack.²⁷

Contraindications to Surgery: Acute myocardial infarction or suspicion of impending infarction precludes operation for at least four to six months. Operation is hazardous in young patients with rapidly progressive symptoms, particularly in those without previous myocardial infarction. These patients are prone to the development of areas of ischemia during or immediately after operation. Electrical instability is prone to occur in these hearts with resultant ventricular fibrillation; an impending medical death becomes a surgical mortality.²²

Cardiac enlargement and congestive failure constitute a relative contraindication to operation. However, in 20 per cent of the patients operated on in this series, the left ventricle was enlarged on fluoroscopy. Only a few had objective evidence of early congestive failure.

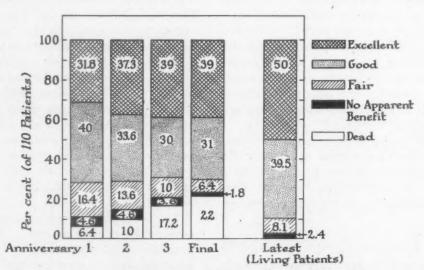


Fig. 1. Clinical status at anniversary of 110 patients after Beck operation. Evaluation is shown for anniversaries 1, 2 and 3. "Final" refers to latest evaluation available (at third, fourth or fifth anniversary). "Latest" refers to evaluation of the eighty-six patients alive three to five years after operation. In the first four columns, "dead" is the cumulative mortality for three to five years.

Although it is too late for much benefit in these instances, the heart is remarkably stable and these patients tolerate operation quite well.

Severe hypertension or any other associated disease limiting life expectancy contraindicates operation. A moderate degree of blood pressure elevation was present in 25 per cent of those

patients who were operated on.

Statistical Evaluation: To comprehensively present the statistical material in this study, the "anniversary method" has been used²⁹ (Figs. 1 and 2). This method employs a principle similar to the actuarial in computing the per cent of survivors for each year after the operation, and in addition, subdivides the surviving patients according to status. Reasonably full use is made of the available data, and a more or less complete answer is provided (Figs. 1 and 2). The percentage of survivors at a stated number of years after operation is an estimate of the probability of the patient surviving an equal number of years.

POSTOPERATIVE CLINICAL COURSE

Generally, following operation, two distinct stages of improvement may be identified: stage 1: improvement in this stage and its duration is variable—weeks or months; and stage 2: this stage of improvement may be imperceptible in onset (overshadowed by the effects of stage 1). Stage 2 may not be apparent until weeks or months after the operation. Usually, stage 2 reaches its peak three to twelve months after operation. In most patients, the transition from stage 1 to stage 2 is poorly defined; however, in many patients, definite clinical manifestations mark this transition.

Following operation, as an expression of the relative influence of these two stages, the clinical course may show immediate or delayed improvement. The early apparent improvement in stage 1 may be non-specific. Although objective evidence of benefit may be found (improvement in the electrocardiogram, "normalized" ballistocardiogram), this stage may be profoundly influenced by various non-specific influences: (1) the "alarm reaction" associated with major surgery; (2) extended bedrest and careful medical supervision (especially from imposing personalities); (3) reflex changes; (4) alterations in peripheral blood flow; and (5) profound psychologic factors (many patients are, no doubt, pleasantly surprised to awake and find that they had even survived the operation).

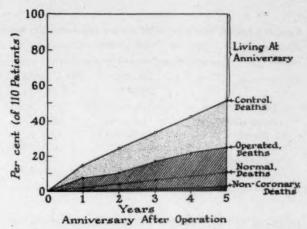


Fig. 2. Anniversary status of 110 patients three to five years after the Beck operation for coronary heart disease. Evaluation as in Figure 1. "Control deaths" are approximate and based on author's control series, and as influenced by similar reported studies. "Operated deaths," one month to five years after operation, presumably due to coronary heart disease. "Normal deaths" refers to life expectancy in the normal population (i.e., those without evidence of clinical disease) of persons in similar age group as "operated" and "controls." "Non-coronary deaths," are patients who underwent operation but died of non-coronary causes.

Stage 1 after operation expresses the interaction of such influences, superimposed on the early surgically-induced improvement in coronary blood flow.

POSTOPERATIVE RESULTS

At present, the 110 patients reported on herein may be divided into three groups according to the length of the follow-up period: (1) approximately five-year follow up, twenty-nine patients; (2) approximately four-year follow up, seventeen patients; and (3) approximately three-year follow up, sixty-four patients.

In a few late postoperative deaths, the exact circumstances of death could not be determined; however, we have assumed such deaths to be caused by coronary disease. In at least three instances death was not a direct result of coronary heart disease.

CLINICAL STATUS

Of the 110 patients discharged from the hospital three to five years ago, twenty-four (22 per cent) have since died. The eighty-six living patients have been classified as follows: class 1, excellent: the patient has little or no pain, is able to work full time and has had no subsequent heart attacks; class 2, good: the patient has occasional heart symptoms on exertional or emotional stress which are readily

Table II

Present Clinical Status of 110 Patients Operated On for
Coronary Heart Disease

	Class	No. of Patients	Per Cent of Series	
	Excellent	43	39.0	
	Good	34	30 8	
	Fair	7	6.4	
	No benefit	2	1.8	
	Dead	24	22.0	

relieved by administration of nitroglycerin or by rest. The patient is able to perform considerably more work than preoperatively; class 3, fair: the result is equivocal with little apparent improvement. The patient is still limited by symptoms. He may have had one or more moderately severe heart attacks since operation; class 4, no apparent benefit: early benefit (stage 1), if it has occurred, has not been sustained. The patient may have had one or more severe subsequent heart attacks.

Of the eighty-six living patients, forty-three (50 per cent) are rated excellent; thirty-four (39.5 per cent) good; seven (8.1 per cent) fair; and two (2.4 per cent) no apparent benefit (Figs. 1, 2 and 3, Tables II and III). Approximately 90 per cent of the living patients achieved a highly satisfactory result and maintain such benefit three to five years after operation. In these 90 per cent the effects on economic productivity in percentages are as follows: (1) able to work with no limitations, 48 per cent; (2) able to work with some limitations, 42 per cent. Classification with respect to pain in the heart during normal activity is as follows: (1) little or no pain, 45 per cent; and (2) much less pain than previously, 45 per cent.

The eighty-six patients alive at this date reached a given level of improvement one to two years after operation and most patients maintained a relative plateau. In some, even two to four years after operation, there was considerable apparent change in the patient's status. There was considerable improvement in seventeen patients; in six there was significant deterioration, beginning two to four years after operation (Figs. 1 and 2).

Ultimate evaluation is concerned exclusively with long term results (stage 2), with only incidental consideration of the early status (stage 1). The most recent evaluation of the original 110 patients is shown in Table II.

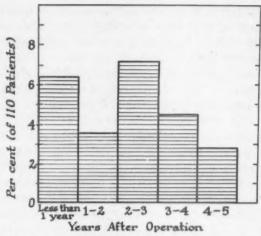


Fig. 3. Late mortality after the Beck operation. Yearly mortality after operation based on the twenty-four late deaths up to five years after operation.

LONGEVITY

Operated Group: The twenty-four late deaths (22 per cent of 110 patients) occurred two months to four years after operation (Fig. 3). Seven patients did not reach their first anniversary; four did not reach their second; eight did not reach their third; four did not reach their fourth; and one did not reach the fifth (Table III).

Of the seven patients who did not survive the first year, four had considerable apparent improvement before death. Of the additional four who died before their second anniversary, two showed considerable improvement before death.

Of the thirteen patients who died two to four years following operation, at least ten

TABLE III

Long Term Mortality After the Beck Operation for Coronary Heart Disease

Anniversary Not Reached	Actual Number of Deaths	Mortality (%)	Cumulative Projected Mortality* (%)
1	7	6.4	6.4
2	4	3.7	10.0
3	8	7.3	17.3
4	4	3.7	21.4
5	1	0.9	24.6
Total at 5 years	24	22.0	24.6

^{*} Anniversary method.

Table IV
Summary of "Objective" Manifestations in Patients Operated On for Coronary Heart Disease

Objective Consideration	Observations After Operation	Interpretation
Blood pressure	If elevated preoperatively, often de- creased after operation	Tendency toward increased peripheral resistance in patient with coronary heart disease may compensate for in-
Peripheral arterial pulses Skin temperature	If absent or reduced in extremity, im- provement after operation If decreased preoperatively, return to normal after operation	creased coronary resistance. Reduced resistance after operation indicates improved myocardial perfusion ²⁵
Shoulder-hand syndrome	Present before operation in 12 patients; improved after operation	This syndrome is related to coronary insufficiency. Improvement after operation indicates more adequate coronary flow
Neurologic manifesta- tions; paresthesias; impaired vision and cerebration	After operation: improved memory, clarity of thought, improved vision	Related to compensatory attempt to di- rect more of cardiac output to coro- naries
Thyroid activity	Patients with thyroid ablation (I ¹³¹) tolerate increased basal metabolic rate (thyroid medication) better, without pain, after operation	Improved oxygenation of myocardium allows increased metabolism
Heart size (x-ray evidence) Congestive heart failure	No increase after operation. If enlarged preoperatively, occasionally decreased after operation Generally, contraindication to operation.	No evidence of deleterious influence of operation. No further damage to heart. Apparent decrease in size of heart and congestive failure reflects
,	Approximately 25 per cent of patients have "early" failure; many improve after operation	improved myocardial contractility af- ter operation
Electrocardiogram	Improved response to exercise tolerance test. Chronic arrhythmias abolished or reduced	After operation, "objective" evidence of improved myocardial oxygenation
Ballistocardiogram	Most patients with abnormal preopera- tive ballistocardiogram eventually show improvement after operation	Improved ballistocardiogram indicates improved myocardial contractility ²⁴

showed significant improvement for a considerable period of time before death. The late deaths occurred in those patients who were considered "salvage" at the time of operation. Little correlation was found between the patient's apparent postoperative status and the occurrence of death.

Control Group: As previously mentioned, in addition to the 110 patients operated on, an equal number during this period were also considered acceptable after preliminary evaluation but were not operated on for a variety of reasons. It is anticipated, after more complete

evaluation, that such patients will serve as an adequate control group. Longevity in the control group has been considered from the approximate date of operation (or non-operation), so that both groups may be comparable.

Preliminary evaluation of longevity statistics in this control series reveals a remarkable degree of similarity, as compared with many similar longevity studies in patients with coronary heart disease. In the first year following the original consideration for operation the control patients who did not undergo surgery had a mortality rate of 15 per cent; and for

each year thereafter (up to five years), 9 per cent per year (Fig. 2).

Also to be considered is the anticipated survival rate for the normal population (i.e., persons in the same age group with no evidence of coronary heart disease). Generally speaking, the five-year survival rate for such a population is approximately 90 per cent.

Probably the most comparable "control" study is that of Lindgren,²⁸ in which the four-year survival rate of the control subjects was 53.8 per cent as compared with the four-year survival rate of the patients who underwent sympathectomy.

OBJECTIVE RESULTS

Unfortunately, the nature of coronary artery disease is such that so-called objective methods for evaluation of medical or surgical treatment are of little value. Reliance on the electrocardiogram or ballistocardiogram is unrealistic. Generally speaking, each patient serves as his own control. In Table IV are summarized certain objective findings in patients who underwent operation.²⁴⁻²⁶

Electrocardiogram: Serial electrocardiograms have been obtained in all patients before, during and at regular intervals following surgery. Postoperatively, there are the characteristic changes of pericarditis, with eventual return to the preoperative pattern. Electrocardiographic evidence of myocardial regeneration is not to be expected after operation; however, in less than 20 per cent of the living patients, electrocardiographic evidence of further muscle destruction is present even five years after operation.

Early in this series, standard exercise tolerance and anoxemia tests were performed in an attempt to evolve a satisfactory objective method for evaluation even in those with evidence of previous myocardial infarction. The lack of correlation between these tests and the status of the coronary circulation has been discouraging. Furthermore, since the operative procedure produces a permanent pericarditis, S-T segment changes following exercise are difficult to evaluate. During the follow-up period, there was excellent correlation in many patients between changes in the exercise tolerance tests

Ballistocardiogram: The ballistocardiogram appears to provide objective confirmation of the

and clinical improvement.

clinical results. In a series of patients undergoing operation, displacement, velocity and acceleration studies, with long term follow up, have been carried out.²⁴ In an earlier series of seventeen consecutive patients, fourteen had ballistocardiographic abnormalities before operation. One to four years after operation, significant improvement associated with impressive clinical benefit occurred in eleven patients.

Two patients had little or no improvement. In three patients whose preoperative ballisto-cardiograms were essentially normal, there was no significant change. Although this ballisto-cardiographic study in a relatively small series of patients is preliminary, it appears to confirm the impressive clinical results in patients operated on for coronary heart disease. The changes in the ballistocardiogram reflect the improved contractility of the myocardium in a great majority of the patients who underwent operation.²⁴

Cardiac Arrhythmia: In many patients, a chronic arrhythmia associated with coronary heart disease was a prominent feature of the patient's symptoms. Although such hearts exhibited increased irritability as a manifestation of impaired coronary arterial perfusion, trauma (in the form of epicardial abrasion) was tolerated remarkably well. In 85 per cent of such patients, a favorable influence on the arrhythmia was associated with ultimate clinical improvement in other symptoms of coronary heart disease.

COMMENTS

The purpose of this report is to attempt to answer the following question: "In view of the admittedly meager improvement in blood supply to potentially ischemic areas of the myocardium, how can we reconcile this with the good clinical results of operation?"

To comprehend this, consideration must be given to the critical influence of relatively slight alterations in blood flow. In many conditions, relatively slight alterations may have profound influence on the clinical status of the patient. Surely, in the surgical treatment of stenotic valvular disease, one cannot help being impressed by the considerable clinical and hemodynamic improvement associated with only partial restoration of the normal valve area. Similarly, in many forms of occlusive or stenotic vascular disease, any

procedure which even slightly improves blood flow may provide gratifying clinical benefit.

MECHANISM DEATH

The majority of deaths associated with coronary disease are the so-called mechanism deaths. Because of an alteration (however slight) in a precariously compensated coronary circulation, a difference in oxygenation of contiguous areas of myocardium results in electrical instability and ventricular fibrillation.22 Under slightly altered conditions, the heart might well have been able to maintain its coordinated beat indefinitely. Total coronary inflow may still be adequate, but distribution is not. As a matter of fact, uniform reduction in total inflow is well tolerated. It should be emphasized that coronary pain (angina) and mechanism death result from unequal distribution, and not necessarily from overall reduction in inflow.22 Marked total reduction in coronary inflow may be well tolerated if intercoronary channels provide uniform distribution of available blood. Only in a minority of coronary deaths does the heart eventually give way because of extensive myocardial degeneration. In these patients, total coronary inflow is reduced to such a point that, even with adequate distribution, myocardial perfusion is inadequate.

The wide variation in response to a given degree of coronary stenosis is influenced by many factors, as yet poorly understood. A significant consideration, no doubt, is a hereditary predisposition to the formation, or activation, of collaterals. For instance, with a given degree of coronary artery stenosis, an adequate collateral response may develop or be activated in a particular patient so that he (and perhaps other members of his family) may never present symptoms of coronary insufficiency, despite multiple complete coronary artery occlusions. On the other hand, in another patient (perhaps coming from a "coronary" family), coronary heart disease appears to run a fulminating course.

CORONARY REACTIVITY

In response to a variety of physiologic and pharmacologic influences, and in common with other organ systems, the coronary vascular bed exhibits a great degree of reactivity.²³ Hypoxia, or more specifically, myocardial ischemia, is the primary physiologic regulator of such activity.³⁰⁻³⁷ Even in the presence of coronary

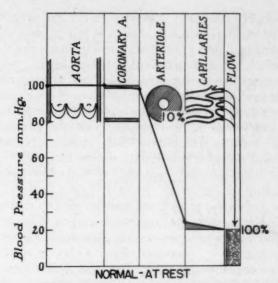


Fig. 4. Stylized graph demonstrating flow-pressure relations of a normal coronary arterial bed. The pressure bed (in mm. Hg) in each component part of the circulation is represented by the heavy line. (From: Brofman, B. L. and Beck, C. S. J. Thoracic Surg., 35: 232, 1958.²³)

arterial sclerosis, such reactivity is still an important determinant of coronary blood flow.²⁸ That such reactivity has profound clinical significance is readily demonstrable in a variety of circumstances.

In order to adapt itself most efficiently to rapid and considerable alterations in its work load, the heart exhibits certain unique features. However, under given circumstances, these very characteristics may have an over-all deleterious influence.²³

The coronary circulation may be characterized as a high resistance circuit, in which, merely by an appropriate reduction in vascular resistance, large increases in blood flow to the myocardium are achieved (Fig. 4). In a sense, the major coronary arteries serve as relatively passive, wide bore tubes with a cross sectional area normally capable of accommodating tremendous increases in blood flow (five to ten times control levels) without significant increases in perfusion (aortic) pressure. For the most part, the sum total of peripheral resistance is normally determined at the level of the arterioles and small terminal arteries. mediated by metabolic, hormonal and nervous influences, changes in the smooth muscle of these vessels regulate the bore of the coronary bed.28

As shown in Figure 4, perfusion of normal coronary arteries from the aorta (first column) is accomplished without loss of pressure head

(second column); the cross sectional area of the major coronary arteries is not a determinant of flow at this level. It is primarily at the arteriolar and terminal arterial level (third column) that coronary blood flow is normally determined. Regulation of flow through the relatively passive capillary bed obtains in the form of an abrupt reduction of the pressure head at the precapillary level. A comparatively small pressure gradient across the capillary bed (fourth column) provides normal flow (fifth column).

Normally, at rest, myocardial oxygen extraction is so high that increased oxygen consumption by the heart, as during exercise, is achieved primarily by increased flow. This is readily accomplished by a decrease in arteriolar resistance so that the pressure head arrives at the capillary bed at a higher level. A twofold increase in the arteriolar bed results in a fourfold increase in flow with no significant alteration in perfusion pressure or in cross sectional area of major coronary arteries. At extremes of physical exercise or when occlusive disease interferes with coronary flow, an increase in oxygen extraction provides an important compensatory mechanism.²³

A careful retrospective evaluation of our studies and other published reports,^{31,38} reveals a heretofore overlooked, but potentially significant, observation. The increase in coronary blood flow, associated with various forms of coronary vasodilator activity, is almost invariably followed by a "rebound" or "dip" phenomenon,^{31,38} so that coronary blood flow is then below normal for extended periods of time. It is merely suggested that such a period of relative vasoconstriction following a period of increased blood flow may have clinical implications

THE COLLATERAL CIRCULATION

Although the excellent studies of Gross³⁹ and of Spalteholz⁴⁰ had demonstrated the anatomic features of intercoronary arterial communications, the criterially decisive role of the collateral circulation in protecting the myocardium was not established until the work of Blumgart, Schlesinger and coworkers.^{41–44} These studies have shown that with adequate collaterals to provide uniform distribution via a final common pathway the integrity of the myocardium may be maintained almost indefinitely, despite multiple complete coronary artery occlusions.

It is significant that such procedures as the

Beck I operation increase collateral circulation, even in the absence of significant pressure gradients. Should there be acute arterial occlusion, such increased collateral circulation, provided in advance, will maintain viability of the myocardium, even though insufficient to maintain contractility. The eventual increase in collateral flow to this low pressure bed then restores contractility so that there is no destruction of heart muscle.

There is little doubt that chronic coronary artery stenosis, with resulting myocardial ischemia, is the stimulus in the activation of intercoronary arterial communications.^{41–44} However, in view of the exorbitant mortality associated with this disease, the assumption that in patients with clinical manifestations of coronary heart disease (and myocardial hypoxia) adequate "natural" collaterals are diligently developing is unrealistic, especially since it implies that the need for further definitive therapy is obviated.

In the presence of adequate collaterals, a most remarkable degree of protection is afforded in man. 41,43,45,46 When controlled, graded coronary occlusion is carried out in dogs, the development of an adequate collateral circulation enables the animal to survive total occlusion, and may prevent myocardial infarction.

SUMMARY

This report is based upon a long term evaluation of more than 600 patients with coronary heart disease operated on by Dr. Claude S. Beck in Cleveland since 1951, with special reference to a more detailed analysis of a series of 110 consecutive patients surviving operation in the two-year period, 1954 and 1955.

Long term follow up of these 110 patients reveals 6.4 per cent died within the first year after operation, a total of 10 per cent had died within two years, and, projected to the fifth anniversary, there was a cumulative mortality of less than 25 per cent (which is approximately 50 per cent of the anticipated mortality in such patients). Of the patients now alive, the condition of 50 per cent is rated excellent, and of 39.5 per cent, good. Almost 90 per cent have achieved and maintained a highly satisfactory result.

APPENDIX

Case Reports

Since, by its very nature, coronary heart disease defies "objective" evaluation, a mere statistical analysis of the results of the operation leaves much to be desired. Only by presenting a series of representative case histories is it possible to provide a reasonably accurate clinical picture of the results of operation. The following case histories have been selected to illustrate various aspects of the influence of the Beck operation on the "natural history of coronary heart disease."

CASE 1. Six years prior to operation, at the age of thirty-nine, following the birth of her third child, hypertension was noted for the first time in this housewife. A sustained blood pressure of approximately 180/100 mm. Hg persisted until a sympathectomy was performed three years later. Since then, her blood pressure has remained at 130/80 mm. Hg. Six months after sympathectomy, the onset of typical angina pectoris on effort was noted. An acute posterior myocardial infarction occurred at the age of forty-three, following which she had progressive incapacity as a result of pain in the heart. At the time of operation, at the age of forty-five, she had been a more or less complete invalid for at least one year.

Following operation there was almost immediate and complete relief from her previously severe pain in the heart. During a follow up of three years to the present time, she has continued to be completely asymptomatic, and has been able to perform all of the various tasks of a mother and a housewife. Her blood pressure has remained at 120/80 mm. Hg.

Case 2. This patient was a forty-eight year old woman, recently widowed, who had suffered at least two massive myocardial infarctions. The first, an anteroseptal, had occurred three years prior to operation; the second, a posterior, occurred eight months prior to operation. Although she had been relatively asymptomatic between the two heart attacks, following the second, severe angina pectoris developed progressively. Although at first fairly mild in nature, for the six months prior to operation her condition had deteriorated to the point at which, even though spending the greater part of each day in bed, pain was almost continuous. She had been forced to take large amounts of narcotic drugs to obtain some relief from pain.

She tolerated the operation remarkably well, and at present, almost five years after operation, continues to be asymptomatic. She has her own jewelry business and continues to work twelve to sixteen hours per day without any symptoms.

Case 3. This patient was a thirty-nine year old man who owned a flower shop. The onset of coronary heart disease occurred approximately three years prior to operation, at which time he suffered a massive posterior myocardial infarction. Following this acute infarction, it was noted that, although

the blood pressure and pulses in the right arm were normal, the blood pressure was unobtainable in the left arm and the left brachial and radial artery pulses were not palpable. For the three years prior to operation, he had progressively severe angina. At first his symptoms were readily relieved by administration of nitroglycerin, but for the six months prior to operation, nitroglycerin had become ineffective. Since his original heart attack, frequent premature ventricular systoles were also a source of great annoyance to the patient. During his stay in the hospital, prior to operation, continued attempts were made to record the blood pressure in the left arm, or to palpate left arterial pulses, but to no avail. After operation the blood pressure and arterial pulsations in the left arm were found to be normal. The premature ventricular systoles have also been abolished.

Although the patient had been unable to work for approximately two years prior to operation, within two months after operation he had returned to work on a full time basis; and he has continued to do so up to the present time with the exceptions to be noted.

Two years after operation, having continued to be almost completely asymptomatic, he suffered a severe "heart attack." The patient stated that the symptoms were exactly the same as his original heart attack, if not worse. The patient was immediately hospitalized, and within twenty-four hours was free of pain. Serum transaminase levels and serial electrocardiograms showed no evidence of acute myocardial infarction. After two weeks of hospitalization, he was discharged, and within a week had returned to full time work. He had no further symptoms until two and a half years after operation, at which time he suffered a similar attack. He was again hospitalized, and results of studies were essentially negative with respect to acute myocardial infarction, and again he returned to full activity. His electrocardiogram up to the present shows no evidence of deterioration, as compared with his preoperative studies.

Case 4. This patient, a forty-four year old man who owned and operated a service station, had suffered an acute myocardial infarction one year prior to operation. A second attack occurred five months prior to operation. Although he had only mild angina prior to the second episode, this time, when he returned to work, he found that he could only work a few hours a day. He took ten to twenty nitroglycerin tablets daily with only partial relief. Two months prior to operation, he was forced to turn over his service station to a relative.

Six weeks after operation, he returned to part time work at his station; and within a few months he was working full time (twelve to sixteen hours per day). He continued to be relatively asymptomatic until two and a half years after operation, at which time Friedländer's pneumonia developed. Death suddenly occurred three days after hospitalization.

Pneumonia was considered the primary cause of death. His coronary status was considered to be more or less unrelated to this final episode.

CASE 5. Approximately seven and a half years prior to operation, this forty-six year old male social worker began to suffer from typical angina pectoris. His symptoms were rather mild until three years prior to operation, at which time he suffered an acute myocardial infarction. He was able to continue his work until one year prior to operation, when he was hospitalized for eight weeks for a massive myocardial infarction. Following this attack, he became completely incapacitated. For six months prior to operation, his symptoms kept him from even venturing out of his house. It was also obvious that this man was suffering progressively severe mental depression. It was thought his emotional state was a direct consequence of his incapacity. During this period his heart had become enlarged, and orthopnea had developed. Severe pain on the slightest exertion was now associated with extreme fatigability and other symptoms of congestive heart failure. In view of the extensive irreversible muscle damage, operation was considered ill advised. However, he pleaded so insistently that, contrary to our better judgment, operation was carried out. He tolerated this remarkably well; prior to his discharge from the hospital, twelve days after operation, he demonstrated an improved sense of well-being, associated with marked diminution in his symptoms.

Within a few months after operation, he had returned to full time activity. In addition to the fact that pain in the heart had been abolished, evidence of congestive heart failure also regressed. Four years after operation, his heart size appears smaller than before operation. He no longer has any manifestations of congestive heart failure and has not required mercurial diuretics since operation. He has not missed a day's work in the past two years, and only on occasion does he have angina, which readily responds to administration of nitroglycerin.

Case 6. This patient was a thirty-six year old truck driver. History revealed that his mother, father and many male and female ancestors had suffered acute myocardial infarctions and death prior to the age of fifty. The patient was relatively asymptomatic until three and a half months prior to operation, at which time he suffered an acute posterior myocardial infarction. Following discharge severe angina pectoris developed so that even slight activity caused pain. For the two months prior to operation, he had become completely incapacitated; his angina became most severe. His clinical course appeared to be rapidly downhill. During operation, his heart was extremely irritable. At times it appeared that he was on the verge of ventricular fibrillation. Operation was finally accomplished; but it was felt that he might not live to leave the hospital.

In two weeks immediately postoperative, there was

a considerable diminution in his symptoms, so that at the time of discharge he had only mild pain on exertion. Within a month thereafter he had returned to full time work driving a heavy tractor trailer. Since such a job entailed extreme physical effort at times, particularly in loading and unloading, he was urged to find a more sedentary job. However, the patient said that this was the only job he knew and he was determined to continue. For a year following the operation, he appeared to be getting along well. However, one day as he was assisting in heavy loading onto his truck, he complained of severe pain in the chest and suddenly fell dead.

At autopsy, no lumen was apparent in any of the major coronary arteries. All of his major coronary arteries were chronically and completely occluded, with no evidence of recent hemorrhage or thrombosis

Case 7. Three years prior to operation, this patient, a fifty-one year old coal miner, suffered an acute myocardial infarction. When he returned to his work, he soon found that angina on only moderate exertion reduced his productivity. He was given a part time sedentary job, which entailed a reduction of about 50 per cent of his income. For a year prior to operation, pain had become so intense that he was unable to carry out even sedentary work. For a few months prior to operation, he was completely incapacitated.

Forty days after operation he went back to his sedentary job. Two months thereafter he asked to return to hard labor; and, although the mine operators were extremely apprehensive, the patient is now working full time at his previously economically productive job. Four and a half years following operation, he claims to be completely asymptomatic.

CASE 8. This patient, a fifty year old salesman, had suffered at least three "heart attacks" prior to operation. Prior to his first attack, two years before operation, he had been completely asymptomatic. A prolonged period of physical and emotional stress (four weeks) antedated his first attack, and a definite cause and effect relation was ascertained. When first evaluated for operation, after his second attack, it was believed that surgery was contraindicated because of the extensive damage to the heart. Subsequently, he suffered a third attack, and signs of early congestive heart failure developed. In view of his complete invalidism and the patient's insistence that he had nothing to lose, operation was agreed on, again reluctantly. Despite extensive heart damage, he tolerated operation remarkably well, and his early postoperative course was uneventful.

Immediately following operation, his clinical condition improved to the point at which, three months after operation, he was able to return to full time work as a salesman. By roentgenogram the heart appeared smaller than it was preoperatively.

He continued his full time employment with little or no symptoms until four years after operation, at which time he died suddenly at work.

Case 9. A thirty-nine year old man who owned a photography store had a history of at least five years of sustained hypertension (175/100 mm. Hg). His family history revealed early invalidism or death from coronary heart disease and hypertension in most of his male ancestors. One year prior to operation, he had suffered an acute extensive anterolateral myocardial infarction. This was complicated by peripheral venous thrombosis, and a diagnosis of thromboangiitis obliterans (Buerger's disease) was considered likely. Prior to operation, confronted by severe angina and great anxiety, he was able to perform only part time work. Although severe hypertension is considered to be a contraindication per se, operation was carried out. His convalescence was complicated by further episodes of peripheral venous thrombosis. Prior to discharge from the hospital, his blood pressure was still at the same high level of 190/120 mm. Hg.

After his return to full time work, the patient became aware of an increased sense of well-being; and he suffered little or no discomfort, as compared to his previous symptoms of coronary heart disease. Approximately six months after operation, it was noted that his blood pressure had returned to normal (130/80 mm. Hg), at which level it has remained all during his five-year follow-up period. There has has been no cardiac enlargement, or any symptoms of congestive heart failure.

Case 10. This patient, a fifty-four year old taxi driver, had a two-year history of angina pectoris. One year prior to operation he suffered an acute anterolateral myocardial infarction and was hospitalized for eight weeks. During this period he was kept at rigid bed rest. Following discharge from the hospital, it was noted that both shoulders had become "frozen." He became completely incapacitated, not only as a consequence of severe pain in the heart, but also because he could not raise his arms more than 45 degrees. The pain associated with this was so intense that physiotherapy and other measures to increase his range of motion failed completely. While under anesthesia for coronary heart surgery, it was ascertained that there was a considerable range of motion possible in both shoulders. Within a few days after operation, active and passive exercises were begun in an attempt to restore

A few months after operation the patient had become completely asymptomatic with respect to his previous cardiac symptoms; with little or no difficulty he was able to move his arms and shoulders through a full range of motion. Three years after operation the patient continues his full time work with only occasional angina on exertion.

Case 11. A sixty-four year old factory worker had been able to continue his work at a punch press despite symptoms of coronary heart disease for at least six years. Presumably he had suffered an acute myocardial infarction six years prior to operation, but had little or no disability after return to work. One year prior to operation, he suffered another attack, an acute posterior myocardial infarction, following which he was again able to return to his previous work. However, beginning six months prior to operation, his symptoms progressed rapidly to the point at which, two months prior to operation, he had become bedridden and had to be fed on occasion because of severe pain on exertion. In view of his precarious condition, operation was considered hazardous. A week prior to operation, even at rest, his electrocardiogram showed, in addition to the evidence of the old myocardial infarction, marked S-T segment deviations.

Anesthesia was induced with extreme care. The heart was extremely irritable and it was feared that ventricular fibrillation might begin at any moment. The situation appeared so precarious that only a modified Beck operation was carried out. Remarkably enough, his immediate postoperative course was uneventful, and he was discharged twelve days after operation. He was urged to take at least three to six months for convalescence.

Three weeks after his discharge from the hospital, he was back at half time work in the factory, and within three months he was working full time at the same drill press he had operated before the onset of his severe symptoms. At present, four years after operation, he continues to work full time with little or no discomfort.

Case 12. This patient was a forty year old attorney. Three months after his older brother died suddenly of acute myocardial infarction, he was hospitalized with an acute anterolateral infarction. His hospital course was relatively uneventful, and he was discharged one month after admission, planning to return to his previous occupation as a trial court lawyer. However, within a few days of his return to full time activity, he suffered another attack, which, according to the patient, was similar to his first. As with his previous attack, the electro-cardiogram showed temporary T wave changes, with return to normal after a few days. He was discharged four weeks after this episode, and was cautioned against returning to his previous work. However, before he could return to part time work, he suffered a third heart attack, similar to the previous two. Again he was hospitalized, again was given the usual treatment and, at the time of discharge, was strongly urged to pursue some other type of work. He became extremely depressed. As he later confessed, he contemplated suicide. Over the strenuous objections of his family physician, operation was performed.

The immediate postoperative course was uneventful; and within six weeks of discharge he had returned to at least part time practice in his office. For the past three years he has been active in trial court and appears to be entirely asymptomatic.

CASE 13. Over the strenuous objections of his office associate (a cardiologist), operation was performed on this fifty-five year old physician with a five-year history of coronary heart disease (in spite of evidence of extensive myocardial damage). There had been at least three episodes of acute myocardial infarction, the last two years prior to operation. At the time of onset of his symptoms of coronary heart disease, he attempted to curtail his general practice, but soon found himself overwhelmed by work. After the second attack, he had become a partial invalid. After the third, he had to give up his practice entirely and he and his family had to subsist on a meager income. Having been informed of the almost prohibitive risk and of the remote chances for much benefit, the patient chose to undergo the operation. He tolerated surgery quite well (at operation, even more extensive damage than anticipated was found), and his convalescence was remarkably uneventful.

A few weeks after his return to his home in Canada, he arranged to carry on a small part time practice. In the three-year period since operation, his practice has grown and he is now able to maintain a load of approximately 75 per cent of his previous capacity (as compared to zero before operation).

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Acute Myocardial Infarction in a City Hospital

IV. Clinical-Pathologic Correlations*

Benjamin A. Rosenberg, m.d. and Monte Malach, m.d. Brooklyn, New York

THE PRESENT report is concerned with sixty-four patients with acute myocardial infarction who came to autopsy at the Kings County Hospital between July 1, 1954 and June 30, 1955. During this period there were 5,000 admissions and 1,000 deaths on Medical Service B, a mortality rate of 20 per cent, with an autopsy rate of 50 per cent. The sixty-four patients in this series represent a necropsy rate of 61 per cent. The clinical features, experiences with anticoagulants and treatment of shock have been reported previously. 38,34,41

PATHOLOGIC FINDINGS

The weight of the heart of fifty-three patients (83 per cent) exceeded 350 gm., which we accepted as the upper limit of normal.⁵ There was evidence of hypertrophy of the left ventricle (thickness more than 15 mm.) in forty-four patients (70 per cent) and of the right ventricle (thickness more than 4 mm.) in thirty-four (53 per cent). Associated old myocardial infarction was noted in twenty-one (33 per cent); fourteen of these were single and seven were multiple.

Transmural myocardial infarction was evident in sixty-one patients, of whom twenty-eight had a single infarct and thirty-three had multiple areas of necroses, a total of ninety-nine individual infarcts. The localization of the infarction according to cases is presented in Table 1. Subendocardial infarction was observed in three additional patients.

Acute thrombosis of the coronary arteries was evident in thirty-seven patients (58 per cent) with two thromboses present in forty-one patients. Five patients had mild to moderate

atherosclerosis; one was not affected. Of the twenty-seven patients who did not have coronary thrombosis, mild to moderate atherosclerosis of the coronary arteries was present in twelve. Two patients had neither acute thrombosis nor atherosclerosis of the coronary vessels.

Pericarditis over the acute process was present in thirteen of the subjects in this study and nine had pericardial effusion. Ventricular rupture was found in three patients; infarction of the papillary muscle was noted in two.

Pulmonary thromboembolism was observed in

TABLE I
Transmural Myocardial Infarction

Localization	Patients (no.)	Per Cent of Series
Single		
Anterior	18	29.0
Posterior	- 6	9.6
Septal	4	6.4
Total	28	45.0
Multiple		
Anterior and septal	19	30.0
Posterior and septal	5	8.0
Anterior and lateral	3	4.8
Anterior, posterior and septal	3	4.8
Anterior and posterior	1	1.6
Anterior, posterial and lateral	1	1.6
Anterior, lateral and septal	1	1.6
Total	33	52.4

^{*} From the Department of Medicine of the State University of New York, College of Medicine, Downstate Medical Center, and the University Medical Service, Service B of Kings County Hospital Center, Brooklyn, New York.

nine patients, in seven it was associated with an anterior myocardial necrosis. There was concomitant mural thrombosis of the right atrium in one patient, combined right and left ventricular thrombosis in one, and left ventricular thrombosis in five. In two patients periprostatic venous thrombi were also seen.

CLINICAL-PATHOLOGIC CORRELATIONS

In the group of twenty-eight with angina pectoris, autopsy revealed cardiomegaly in twenty-two (79 per cent), left ventricular hypertrophy in sixteen (57 per cent) and old infarction in twelve (43 per cent). In the four patients with angina pectoris without evidence of congestive heart failure or hypertension, there was no cardiac enlargement.

In the twenty-five patients with a history of hypertension, cardiomegaly was present in twenty-three (96 per cent), left ventricular hypertrophy in sixteen (64 per cent), right ventricular hypertrophy in fourteen (56 per cent) and old myocardial infarction in twelve (48 per cent).

Those patients with a history of angina pectoris had more extensive and severe sclerosis in branches of all arteries. Curiously, eight patients with a history of old myocardial infarction had a low incidence of coronary sclerosis in all branches, none being noted in the anterior and posterior descending arteries. Significantly, six of the seven patients who did not previously have angina, myocardial infarction, hypertension or diabetes, had severe sclerosis of a branch of at least one major coronary artery, and no evidence of an old infarction.

Pain in the chest of less than one month's duration was a presenting complaint in thirty-seven (58 per cent) of the sixty-four patients in the study. Acute coronary thrombotic occlusions were demonstrated in twenty-four of these (65 per cent). Of the twenty-seven patients who did not have pain in the chest, thirteen (48 per cent) had acute coronary thrombosis; four of these were multiple.

Electrocardiograms were available in forty-seven patients. Old myocardial infarction was diagnosed in five (11 per cent), four were anterior and one was anterior and posterior by electrocardiography. At autopsy, two old infarctions were confirmed, only one by location. In addition, twelve other old infarctions were found in the forty-two patients without electrocardiographic evidence. There was a total of twenty-one old infarctions in the sixty-four patients.

In thirty-four patients, forty-two acute myocardial infarctions were diagnosed by electrocardiogram; thirty (in twenty-four patients) were confirmed by autopsy. In eleven of the twenty-four patients, the infarction proved to be more extensive than indicated by the electrocardiogram. The presence of an old myocardial infarct at necropsy in nine patients did not interfere with the localization of acute infarction in six patients. Six patients with an old cardiac infarction had acute necrosis at the same site.

A study of the ten patients with inaccurate localization of the acute infarction revealed that (1) five patients died less than forty-eight hours after admission, therefore few electrocardiographic studies were available; (2) three of the remaining five patients had associated old myocardial infarction; and (3) in eight of the ten patients in whom the size of the infarct was determined, five infarcts were in excess of 7 cm.

Of the thirteen patients without confirmation of acute infarction, five had complete left bundle branch block, four had associated old infarction involving the same area of myocardium, two died within forty-eight hours after admission, and three patients had an acute infarct of less than 2 cm. in its largest diameter. There was one case of pericarditis and one of complete right bundle branch block. Electrocardiograms of two patients failed to show diagnostic signs or interfering factors.

PATHOLOGIC-CLINICAL CORRELATIONS

The causes of death have been divided into sudden and non-sudden.

SUDDEN DEATH

Sudden death was noted in thirty-nine patients (60 per cent); twenty deaths were presumed to be due to ventricular standstill in the absence of other pathologic causes of death. The size of infarct was determinable in twenty-four of the twenty-nine cases of presumed ventricular arrhythmia and was less than 2 cm. in diameter in four, between 2 and 7 cm. in seven and over 7 cm. in thirteen. Shock was a contributory cause of death in five of the group in whom ventricular arrhythmia was assumed.

There were three instances of myocardial rupture (two women and one man), an incidence of 4.7 per cent of the group who came to autopsy. These observations were made pathologically: (1) the site of rupture was the left ventricle,

two in the anterior wall and one in the posterior wall; (2) there was cardiomegaly in two; (3) the volume of hemopericardium was 200, 300 and 400 cc., respectively; and (4) the acute infarction was of medium size in one and large in two. Clinically, previous hypertension was noted in two patients, only one of whom revealed an elevated diastolic level on admission. Pain in the chest was a presenting symptom in all. In two of the three patients with rupture terminal shock developed. Death occurred on the third, sixth and eleventh day, respectively. None of these patients received anticoagulants, but all were given ascorbic acid.

Pulmonary thromboembolism was demonstrated at necropsy in nine patients (14 per cent). Two of these patients did not die suddenly and will be discussed in the next section of this paper. One patient whose disease was correctly diagnosed antemortem had an acute infarction of the posterior wall visible electrocardiographically, whereas at autopsy a massive anteroseptal process was demonstrated. Mural thrombosis was present in the left side of the heart in five patients and in both sides in one: periprostatic venous thrombosis was present in another. No data on venous occlusions of the legs were available. Infarction of the lung occurred in only two patients and neither patient had pulmonary edema or chronic congestion. Two patients had pneumonitis. The acute myocardial infarct was over 2 cm. in five patients in whom the size could be determined. None of the seven patients with pulmonary thromboembolism received anticoagulants; two had transient shock.

NON-SUDDEN DEATH

Non-sudden death occurred in twenty-five patients. Pathologically, there was pulmonary edema in thirteen patients, chronic passive congestion of the lungs in twelve, non-pulmonary thromboembolism in four, pulmonary thromboembolism in two, atelectasis in two and gastric dilatation in one. The size of the infarct was determinable in thirteen of the nineteen patients with pulmonary edema and/or congestion, and was in excess of 7 cm. in diameter in ten. Clinically, the causes of non-sudden death in the twenty-five patients were shock (thirteen), congestive heart failure (seven), combined shock and failure (three), mesenteric embolism and shock (one) and gastric dilatation and aspiration pneumonia (one).

Both patients with pulmonary thromboembolism who did not die suddenly had pneumonitis of the right lower lobe of the lung with edema. There was no infarction or chronic passive congestion. One had a mural thrombus in the right atrium and the other had periprostatic venous thrombosis. The size of the myocardial infarct was over 7 cm. in one patient and of unlisted size in the other. Neither patient had shock nor received anticoagulants, but both had congestive heart failure.

Fifteen of the patients in these series underwent examination of the brain. Of these, eleven had encephalomalacia. Cerebral arteriosclerosis was of minimal degree in three, moderate in four and severe in three. One patient had cerebral embolism and another had cerebral thrombosis. The patient with cerebral embolism also exhibited pulmonary embolism without infarction. a massive anterolateral myocardial infarct and a mural thrombus in the left ventricle. The patient with cerebral thrombosis had pulmonary edema and chronic passive congestion of the lungs and liver. Of the nine patients with encephalomalacia who did not have cerebral thromboembolism, congestive heart failure was present by history in five, hypertension in two and a cerebrovascular accident in one. On physical examination, one patient was hypotensive, five were normotensive and three were hypertensive. All displayed one or more signs of congestive heart failure. The three patients with hypertension were all shown to have severe cerebral arteriosclerosis at autopsy.

The sixty-four patients on whom autopsies were performed were classified according to the size of the acute infarct. The largest diameter of infarct was thus determined in fifty-one cases. There were five small (less than 2 cm.), fifteen medium (2 to 7 cm.) and thirty-one large (more than 7 cm.). Table II correlates important clinical-pathologic findings with the greatest dimension of the infarct. In general, the patients with large infarcts had the least historical evidence of prior myocardial infarction, congestive heart failure or hypertension. The group with small infarcts had the greatest historical incidence of angina pectoris. Pain in the chest as a presenting symptom was significantly (p < 0.01) more frequent in the group with large infarcts. Three instances of myocardial rupture occurred in patients whose infarcts were more than 2 cm. The group with small myocardial infarcts had the lowest incidence of left ventricular failure, cardiomegaly

and shock on clinical examination, and of acute coronary thrombosis and pulmonary edema at autopsy. There was no cardiac rupture, mural thrombosis or pulmonary thromboembolism in this group.

Mural thrombosis occurred twenty-five times in twenty-one patients, four of whom were given anticoagulants. Six patients with mural thrombi died within forty-eight hours and had not received anticlotting agents. Although three of these six patients died suddenly of pulmonary thromboembolism, the mural thrombi were located in the left side of the heart. As previously noted, mural thrombi occurred predominantly in patients with a large myocardial infarct; one patient had a cerebral embolus presumably from a thrombus occurring in the left side of the heart.

COMMENTS

The observation that all fifty-three cases of cardiomegaly in our series of sixty-four patients with acute myocardial infarction who came to autopsy were associated with ante- or postmortem evidence of hypertension, congestive heart failure or both is supported by several studies. ^{18,46} The incidence of cardiomegaly varies from 50 per cent⁷ to 89 per cent. ³⁰

Multiple acute infarctions in our series occurred in thirty-three of the sixty-four patients. Representative of the literature is the incidence of 41 per cent reported by Wartman and Hellerstein⁴⁵ in 164 patients with myocardial infarction.

Thirty-three per cent (twenty-one) of our patients had one or more mural thrombi demonstrated at autopsy. Hellerstein and Martin²² noted an incidence of 49 per cent in 924 collected cases, including 160 of their own patients. The location of the mural thrombi predominantly in the left ventricle in the patients of our series conforms to the previously known pattern.^{18,29}

Location of Infarction: In general, the anterior wall of the heart is involved two and a half times as often as the posterior wall. This may reflect, in part, the poorer prognesis of patients with infarction in the former location. Thus in our series, the anterior surface was involved in 65 per cent, the posterior surface in 17 per cent, both surfaces in 8 per cent and the lateral surface alone in none; 6.4 per cent were classified as purely septal without designation of anterior or posterior (Table 1). MacDonald and Bentley³¹ found that in a series of 100 pa-

Table II

Correlation of Size of Infarct with Various Factors

	Perce	entage with	Factor
Factor	Small (5)*	Medium (15)	Large (31)
History			
Angina	60	27	48
Myocardial infarction	20	27	6.4
Congestive failure	60	79	45
Hypertension	60	46	38
Presenting with pain in			
the chest	20	33	70
Physical findings			
Left ventricular failure.	40	87	68
Cardiomegaly	40	60	67
Course			
Increased or new fail-		100	
ure	0	27	19
Shock	20	40	42
Sudden death	80	60	61
Death at 0-2 days	40	40	26
Death at 3-7 days	20	20	22
Anticoagulants given	40	0	29
Autopsy findings			1
Cardiomegaly	80	92	83
Mural thrombosis	0	26	45
Old infarction	40	53	29
Coronary thrombosis	20	67	70
Pulmonary thrombo-			7 11
embolism	0	.6.6	16.
Pulmonary congestion	60	53	54
Pulmonary edema	20	60	24
Rupture of myocar-			4174
dium	0	0	6.6

^{*} No. of patients.

tients with acute myocardial infarction who came to autopsy, the locations were: anterior, 65 per cent; posterior, 28 per cent; combined, 3 per cent; and lateral, 4 per cent. None were classified as purely septal. In a series of 164 patients studied postmortem, Wartman and Hellerstein⁴⁵ demonstrated involvement of the anterior wall in 72 per cent and of the posterior wall in 28 per cent.

The relation of septal involvement to infarction of the anterior and posterior wall is worthy of note. In our series, slightly less than half of each group of anterior and posterior infarcts affected the interventricular septum. In one report previously mentioned, 45 one-half of the anterior and one-quarter of the posterior infarcts involved this region, while in another paper 31 the proportions were one-third and one-quarter, respectively.

Location of Coronary Thrombosis: Reports in

TABLE III

Location of Acute Coronary Occlusion

Branch of Coronary	Fin	orn and kelstein ²³ 0 cases)	MacDonald and Bentley ³¹ (39 cases)		ntley ³¹ Helle		Yartman and Professional Profes	
System	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent
Right main (or circumflex)	61	61.0	11	29.0	53	40.0	11	30.0
Posterior descending	414				3	2.0	5	14.0
Right marginal							1	2.7
Left main			2	5.2	5	4.0	7	19.0
Left circumflex	27	27.0	9	23.0	38	22.0	4	11.0
Anterior descending	56	56.0	23	60.0	93	70.0	13	35.0
Ramus primus of anterior descending	15	15.0	* *					
Left circumflex branch to ob- tuse margin	7	7						

the literature have varied as to the location of the coronary thrombosis responsible for the acute myocardial infarction. Data from our patients and from a few representative studies are presented in Table III. On the basis of three collected series, Wood⁴⁹ presented figures of 66 to 75 per cent for the anterior descending branch, 25 to 40 per cent for the right coronary artery and 5 to 33 per cent for the left circumflex. He states that thrombosis of the left main trunk was rare. Thus, it should be pointed out again that the prognosis is poorest in anterior myocardial infarction.

Non-thrombotic and Thrombotic Occlusion: The relation of coronary thrombosis to acute infarction in general, and to its localization in particular, has been the subject of much study. Exemplary of the many studies previously reported, 16,30,49 Wood observed 29 per cent of acute myocardial infarctions without coronary thrombosis based on a compilation of three series, whereas Evans' figure was 57 per cent of 1,358 collected cases representing eight series with a range of 24 to 90 per cent. 16 We observed that two-fifths of our patients did not have thrombotic occlusions. The varying incidence of non-thrombotic occlusion is attributed18,42 to loose adherence of the fresh thrombus to the arterial wall and failure to employ special technics, such as the combined injection and dissection method of Blumgart and Schlesinger. 6,42 We believe that these explanations also hold true for the apparently low incidence of multiple acute thrombi in our series (four of thirty-seven cases) and in those of others. Thus it has been shown that the majority of coronary

thromboses with acute myocardial infarction are multiple. 18,42,49

Acute thrombotic occlusion of a coronary vessel with or without underlying arteriosclerosis might be expected to produce pain. In our series, 65 per cent of the patients with thoracic pain demonstrated thrombotic occlusion, as contrasted to 48 per cent of those without this symptom. Levine and Brown³⁰ observed these proportions to be two-thirds and one-third; respectively. In Yater's series of patients with coronary artery disease who came to autopsy, 7 per cent of those without pain revealed thrombotic occlusion alone as compared to 24 per cent of the remainder. However, the proportions of those with both thrombotic and sclerotic occlusion were about the same in patients with and without pain.

Explanations for the occurrence of myocardial infarction in the absence of coronary occlusion include the failure to employ the combined injection-dissection technic⁴² in examining these vessels. In addition, Boyd⁷ lists the great incidence of hypertension in such cases, a transient fall in the intra-aortic blood pressure and a failure of the mechanism governing compensatory dilation of the coronary arteries. However, in our series there was no significant difference in the incidence of hypertension in the patients with or without thrombosis.

Electrocardiogram: The literature has many reports of correlations of electrocardiographic and postmortem findings.^{1,8,17,25,27,28,36,37,40,43,47} Thus, Levine and Phillips²⁸ reported that only one-fifth of the old infarcts diagnosed by electrocardiography were confirmed by autopsy,

as in our series. Conversely, others^{8,18} have commented on the difficulty of diagnosing old infarction in the presence of an associated acute process.

In three reported series the diagnostic accuracy of the acuteness of the myocardial infarction was 75 per cent,²⁸ 82 per cent⁴⁷ and 82 per cent.²⁵ In the latter study it is not clear as to whether or not the series of 304 cases were all acute infarctions. The diagnosis of acute myocardial infarction by electrocardiography in our thirty-four patients was substantiated at autopsy.

The accuracy of electrocardiographic localization of the acute process in association with an old infarction in our series is similar to that reported by Feil, Cushing and Hardesty.¹⁷ Elimination of the patients with associated remote infarcts raised our accuracy by not more than 6 per cent.

The reasons for failure to correctly diagnose the degree and/or localization of myocardial infarction include:1,18 the coexistence of old infarction, multiple (acute) infarcts, widespread pericarditis, bundle branch block, insufficient number or malposition of chest leads or improper timing of the electrocardiogram, digitalis therapy or anatomic position of the heart (markedly transverse or vertical). Failure to correctly localize the site of the acute infarction in ten of our sixty-four patients was explained satisfactorily in seven by one or more of the aforementioned factors. Failure to diagnose any acute infarction in thirteen other patients of the series was accounted for in eleven by these same reasons.

Causes of Death: Sudden death from presumed ventricular fibrillation, most frequently a diagnosis by exclusion, has been reported in 54 per cent of sixty patients with fatal acute coronary occlusion within six weeks after the acute episode and within four weeks in 26 per cent of 281 patients with myocardial infarction who came to autopsy.88 Our proportion of 45 per cent falls within this range. Myocardial rupture as a cause of death in patients with acute myocardial infarction has been reported in 3.3 per cent of one series 50 referred to previously, and in 6.3 per cent of the other.32 The incidence of 11 per cent for pulmonary embolism as a cause of death in our series compared with those of 6.5 per cent¹⁵ and 10 per cent⁵⁰ reported. Congestive heart failure as a cause of death has been recorded in 35 per cent³² and 26 per cent.⁵⁰ Our proportion of 28 per cent is within this range. Pulmonary infarction without specific mention of pulmonary thromboembolism has been listed as a cause of death in 4.2 per cent of 281 fatal episodes.³² Non-pulmonary thromboembolism as a cause of death in patients with myocardial infarction who came to autopsy has been reported in 1 to 7 per cent.^{32,49,50} In our series of sixty-four patients on whom autopsy was performed, four deaths (6.2 per cent) could be attributed to this cause.

Myocardial Rupture: Reports dealing with this complication of acute myocardial infarction are numerous. 4,10,14,19,24,26,88,39 In general, this event occurs in 1.5 to 3 per cent of all cases of acute myocardial infarction and accounts for about 5 to 13 per cent of fatalities. Somewhat at variance with these figures is the incidence of 19 per cent reported by Levine and Brown³⁰ in a group of forty-six patients with fatal acute coronary occlusion. The proportion in our series (4.7 per cent) is at the lower margin of this range. Classically, this cause of sudden death occurs in patients beyond the age of fifty who remain hypertensive and/or engage in undue physical activity during the first three weeks of the illness. 18,39,49 Our three patients with rupture generally fit the aforementioned clinical characteristics, with the exception that only one patient had hypertension. The hypertension disappeared during her course in the hospital; no abnormal exertion was recorded.

Rupture of the myocardium following infarction is reportedly twice as common during anticoagulant therapy44 and less common when prophylactic ascorbic acid is given.21 The reverse was observed in our series, in which the three patients with this complication had received ascorbic acid (50 mg. daily) and no anticoagulants. In explanation, one might point out the relatively small number of patients in the present series who were treated with anticoagulants. Experience with prophylactic administration of vitamin C is meager,21 and in our patients a controlled study was undertaken. As reported in a previous paper, 33 a statistically significant improvement in the mortality rate was unexpectedly seen in the patients so treated. The study had been originally designed to evaluate supplementary vitamin C administration in preventing myocardial rupture. It may be that such treatment was inadequate to compensate for a poor dietary intake of this substance prior to hospitalization.11

Pathologically, this complication usually occurs in the anterior wall of the left ventricle, in an area not previously scarred^{14,48} and in the absence of severe coronary artery disease. Of our three patients with rupture, none had a previous history of angina or infarction and only one had cardiorrhexis in the site of a previous infarction.

Pulmonary Thromboembolism: This complication was reported22 to occur in 24 per cent of 1,146 patients with myocardial infarction who came to autopsy collected from ten series with a range of 0 to 76 per cent. It was listed as a cause of death in 11 per cent of 577 such cases, with a range of 5 to 46 per cent. In our series, pulmonary thromboembolism was present in nine patients (14 per cent) and was the main cause of death in seven (11 per cent). This lesion accounted for one-fifth of sixty-four instances of sudden death, a finding similar to that in our own series. The origin of pulmonary emboli from non-cardiac sources has been stressed by several workers. 18,49 In only two of our nine patients with pulmonary thromboembolism could mural thrombi in the right side of the heart have been the originating site. Since the veins of the legs were not examined in any of our patients, the latter source cannot be definitely excluded. The presence of pulmonary congestion both ante- and postmortem in only two of our nine patients with pulmonary embolism, neither of whom died suddenly, and the presence of pulmonary infarction in two other patients without evidence of left ventricular failure, are of interest because of the more usual associations reported.7,15 Extensive pulmonary infarction in one series of thirteen patients with pulmonary embolism and associated antemortem congestion of the lungs were demonstrated in all but one.15

Cerebral Thromboembolism and Encephalomalacia: Cerebral thromboembolism^{20,22} in association with or as a complication of myocardial infarction has been described in 7.7 per cent of 851 collected patients who were autopsied. In our series one instance of cerebral embolism and three of thrombosis constitute a combined incidence of 6.2 per cent. In our patient with embolic occlusion a pulmonary embolism and a mural thrombus in the left ventricle were also demonstrated at necropsy. The encephalomalacia in this patient as well as in one of the three with cerebral thrombosis is easily explained. None of the patients with cerebral softening presented with hemiplegia at the onset of the

myocardial infarction, as has been described by others.2,3,9,12,13 Thus, Bean and Read2 recorded eight cases of myocardial infarction which had been diagnosed clinically as acute vascular lesion of the brain in seven patients and as epilepsy in one. At autopsy, advanced cerebral arteriosclerosis was noted in seven patients, but no acute vascular occlusion was observed in any. The explanations² of relative cerebral ischemia superimposed on cerebral sclerosis and additional pulmonary congestion causing further anoxemia are applicable to our material. Of our nine patients with encephalomalacia in the absence of cerebral thromboembolism, severe cerebral sclerosis was present in three, hypertension on admission in one other and one or more signs of congestive heart failure clinically in all.

Size of Acute Infarct: With the exception of electrocardiographic studies, the literature presents few correlations of the size of the myocardial infarct at autopsy with the clinical features. In our series the lower incidence of previous angina pectoris and associated old infarction at necropsy in the group with large infarcts is expected, since there was little opportunity for collateral coronary circulation through chronic ischemia to develop.6 The latter explanation may also apply to the significantly higher incidence of pain in the chest in the patients whose infarct was larger than 7 cm. It is, furthermore, not surprising to find a lower incidence of shock and congestive failure in the group with small infarcts during the hospital

In the early series of Levine and Brown³⁰ there was a range of size of the acute myocardial infarction of 2 to 8 cm., with an average of 3 to 5 cm. In twenty-four of their forty-six patients in whom the onset of the disease was known, no relation was established between the extent of the necrosis and the survival. The sudden death of four of the five patients with small infarcts in our study during the first forty-eight hours is presumed to be due to ventricular fibrillation or cardiac standstill. The fifth patient died from congestive heart failure. Most of the patients with the larger infarcts (in excess of 2 cm.) survived more than fortyeight hours and succumbed primarily to cardiac rupture, thromboembolism and congestive heart failure. We have no explanation for the greater incidence of presumed ventricular arrhythmias in the group of patients in our series with small infarcts.

SUMMARY

1. A correlation of the clinical and pathologic features of sixty-four patients with acute myocardial infarction who came to autopsy in a city hospital during a one-year period is presented.

2. The absence of cardiac hypertrophy in coronary artery disease without heart failure

or hypertension is reaffirmed.

3. The anterior wall was involved three times as often as the posterior wall, reflecting the poorer prognosis of patients with infarction of the anterior wall.

4. Acute coronary thrombosis was observed forty-one times in thirty-seven patients, an inci-

dence of 58 per cent.

5. The acute thrombi were located principally in the anterior descending and right main coronary arteries and were associated with coronary sclerosis in all but one patient.

6. Myocardial rupture was demonstrated in 4.7 per cent, despite administration of vitamin C but without anticoagulant therapy. The established clinical background of myocar-

dial rupture was observed.

7. Pulmonary thromboembolism was observed in 14 per cent, and was associated with (a) pulmonary infarction in only two of the nine patients, (b) the larger myocardial infarcts, and (c) a correct antemortem diagnosis in one patient.

8. Encephalomalacia was noted in eleven of the fifteen patients whose brain was examined and was associated with cerebral thromboembolism in two and with congestive heart failure and

cerebral sclerosis in the others.

9. The size of the infarct was small in 10 per cent, medium in 29 per cent and large in 61 per cent, and had positive correlation with the occurrence of pain in the chest.

10. Mural thrombosis occurred twenty-five times in twenty-one patients, was predominantly left ventricular and was associated with large myocardial infarcts. Only a minority of these patients received anticoagulation therapy.

11. Death was sudden in 60 per cent, being due to ventricular arrhythmia, myocardial rupture or pulmonary thromboembolism, and non-sudden in 40 per cent, being explained principally by shock and congestive heart failure.

12. Electrocardiographic diagnosis of the acuteness of infarction was correct in 72 per cent, but in no case was a tracing completely normal.

13. Accuracy of the clinical localization of the individual infarct was 71 per cent.

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Anatomic and Clinical Significance of Calcification of the Aortic Knob Visualized Radiographically*

IRVING CHAPMAN, M.D. Elmhurst, New York

CALCIFICATION in the wall of the aortic knob visualized radiographically is recognized as being at a site of severe arteriosclerotic changes.^{1,2} Because of the ease of radiographic examination of this area, it would be of interest to determine if this calcification is indicative of the amount and degree of arteriosclerotic change in the remainder of the aorta. To clarify the question, the study reported herein was undertaken.

MATERIAL AND METHODS

Aortas from one hundred consecutive autopsies of patients more than sixty years of age who had a recent roentgenogram of the chest were examined. The unopened aortas obtained at postmortem examination were x-rayed both in the lateral position and in the position which most closely approximated that during life. In the course of the study, it became apparent that further information would be obtained by indicating the ligamentum arteriosum on the x-ray film. This was accomplished by inserting a steel needle through the approximate center of the structure, with the tip apposed to the aortic wall. The aortas were then opened and examined for arteriosclerotic alterations.

After the foregoing examinations were completed, the roentgenograms of the chest taken during life were compared with the postmortem data.

Grading of Arteriosclerosis: Because of the inconsistent distribution of arteriosclerosis in various portions of the aorta, it is an impossible task to accurately grade the over-all degree of arteriosclerotic alterations. Alterations vary in different areas with a low index of predictability. To obviate this confusing factor, the aorta was schematically considered in three parts: (1) thoracic (from the point of origin to the diaphragm); (2) upper abdominal (from the diaphragm to the orifice of the inferior mesenteric artery); and (3) lower abdominal (from the inferior mesenteric artery to the beginning of the iliac bifurcation). These areas

were evaluated for elasticity, plaque formation, atheromata and calcification, and graded from 0 to 4 with 4 indicating the most severe involvement. The criteria proposed by Sjovall and Wihman³ for gross grading of aortic arteriosclerosis were used as a guide.

RESULTS

Calcification of Aortic Knob: In thirty-two cases calcification of the aortic knob was seen in the clinical x-ray film (Table 1). In all these a corresponding calcified arteriosclerotic plaque was demonstrated on the postmortem x-ray film and on gross examination. All of these aortic knob plaques, however, had a most interesting distribution. In the great majority they were either totally or in large part at a site either contiguous to or in the immediate vicinity of the aortic insertion of the ligamentum arteriosum. Almost all the remainder were approximately along the linear designation termed the aortic isthmus (a slight

Table I Summary of Findings*

Finding	No
Arteriosclerotic alteration in aortic knob	99
Calcification in aortic knob (x-ray finding) Calcification in aortic knob (clinical x-ray exami-	91
nation)	32
finding) with minimal arteriosclerosis of entire aorta	19
Significant calcification in aortic knob (clinical x-ray examination) with minimal arteriosclerosis of entire aorta	8

^{*} Postmortem findings except as indicated.

^{*} From the City Hospital at Elmhurst, Elmhurst, New York.

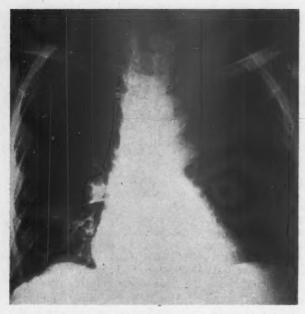


Fig. 1. Thin rim of calcification visualized on clinical x-ray film (from 4 to 6 o'clock of aortic arch).



Fig. 2. Postmortem x-ray film of aorta shown in Figure 1 (with needle in ligamentum arteriosum). Tip of needle abuts on sclerotic patch which is anatomic substrate for the visualized calcification in Figure 1. Gross examination demonstrated only a minimal degree of aortic arteriosclerosis.

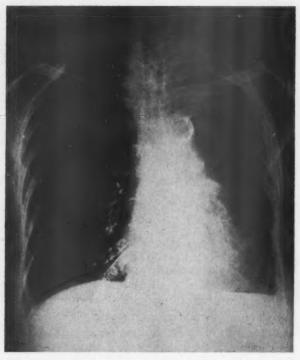


Fig. 3. Clinical x-ray film showing severe calcification of arch of aorta (circular shadow).

constriction at times seen between the origin of the left subclavian artery and the attachment of the ductus arteriosus).

The calcification on clinical roentgenogram appears linear and almost always continuous, and delineates either a portion or the entire circumference of the aortic knob. The anatomic substrate of the x-ray alterations consists of calcified arteriosclerotic plaques which are at times separated and which frequently are in different superimposed anterior-posterior planes.

When calcification is radiographically visualized along the lower segment of the aortic circumference it almost always represents a calcified plaque contiguous to the aortic attachment of the ligamentum arteriosum (Figs. 1 and 2). In four cases the responsible plaque was one-half to three-fourths of an inch proximal to this area. The radiographically visualized calcification of the lateral margin of the aortic knob represents a plaque contiguous to or slightly separated from the one originating at the attachment of the ligamentum arteriosum and most frequently coursing towards the origin of the left subclavian artery. The superior segment of the visualized circumferential calcification derives from a calcified plaque distributed in the main in the vicinity of the mouth of the large vessels taking origin from the aorta.

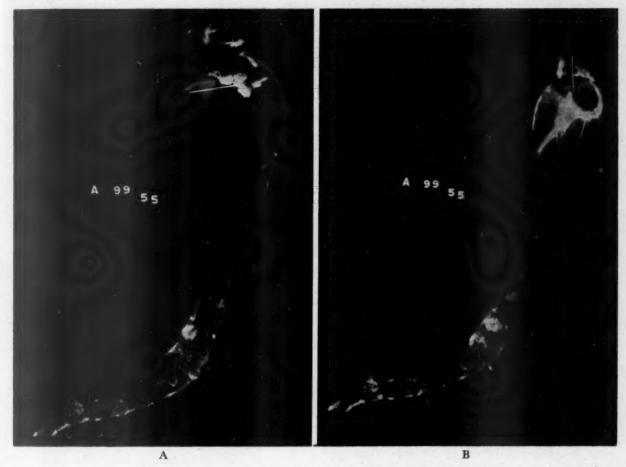


Fig. 4. Postmortem x-ray films (same patient as Figure 3). A, lateral position, demonstrating plaque at attachment of ligamentum arteriosum, other plaques in contiguous areas and at origin of large vessels originating from arch. Thoracic and upper abdominal aortas show minimal calcification. Calcification of the lower abdominal aorta is moderate. B, in position simulating that during life, demonstrating how the separated plaques, when projected in an anterior-posterior direction, become superimposed and are registered as a calcified ring. Arteriosclerotic alterations on postmortem examination were minimal in the thoracic and upper abdominal areas, and slight to moderate in the lower abdominal aorta.

Ninety-nine of the 100 aortas examined postmortem bore a recognizable area of cicatrization, retraction or an arteriosclerotic plaque corresponding to the aortic attachment of the obliterated ductus botalli. Of these, ninety-one showed some calcification in this area on postmortem examination.

Correlation of Arteriosclerosis in Aortic Knob and Remainder of Aorta: The finding of striking importance, however, was that the plaques found in the vicinity of the aortic termination of the ligamentum arteriosum and along the aortic isthmus bore no correlative relation to the degree of arteriosclerotic alteration in the remainder of the aorta. In nineteen of the aortas with minimal arteriosclerosis (grade 0 to 1), there were significant calcified arteriosclerotic plaques in the area of the ligamentum

arteriosum insertion. It was interesting to find that eight of these patients showed a visibly calcified area in the aortic knob on clinical roentgenogram of the chest (Figs. 1 to 4). Stated differently, in eight patients with a calcified plaque visible on the roentgenogram of the chest, minimal arteriosclerosis was seen on postmortem examination of the aorta (except for a "sclerotic patch" present in the vicinity of the ductus botalli insertion). In the other eleven cases the same situation was observed anatomically and on postmortem roentgenogram although calcification was not visualized on clinical roentgenogram (Figs. 5 and 6).

The previous findings of others that there was no consistency in the degree of arteriosclerotic alteration in the various portions of the aorta

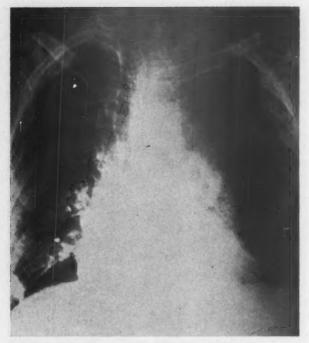


Fig. 5. Clinical x-ray film with no calcification visualized in aortic arch.

are confirmed. In thirty-eight cases there was a somewhat similar degree of alteration throughout the aorta. In sixty-one cases there was a marked increase in severity from the thoracic aorta to the abdominal aorta (Fig. 6). The greatest increment of change was from the thoracic aorta to the upper abdominal aorta with a lesser increment from the upper to the lower abdominal aorta.

In one case there was a slightly greater degree of arteriosclerosis in the thoracic aorta than in the abdominal aorta.

COMMENTS

The area of the aortic arch corresponding to the point of attachment of the obliterated ductus arteriosus is an almost constant site of an arteriosclerotic plaque in the older age group. This site of predilection has been frequently observed in studies of arteriosclerosis and in a recent book4 has been designated the sclerotic patch. This area might vary from a round shallow retraction 2 to 4 mm. in diameter (as more frequently demonstrated in the younger age groups), through all variations of arteriosclerotic alteration until it presents as a firm area of severely calcific arteriosclerosis in the older age groups. In a frequently observed stage of development (usually from the fifth decade on), it presents as an ellipsoid plaque



Fig. 6. Postmortem x-ray film of same patient demonstrating severe calcification of aorta in lower thoracic and abdominal portions. The absence of calcification in the aortic isthmus is reflected in the lack of calcification visualized on the clinical x-ray film (Fig. 5). Entire aorta showed a severe degree of arteriosclerosis on gross examination.

partially or totally calcified with the long axis almost transverse to the aortic lumen (Fig. 7). The surface is slightly irregular and is usually at the same level or minimally below the contiguous surface. The area is frequently delimited from the surrounding aorta and when removed by sharp dissection leaves a large defect in the aorta which most often consists of the major portion of all coats. Frequently this plaque bears a fine, thin ridge across the intimal surface of its long diameter. At times an arteriosclerotic plaque is present at the tip of this sclerotic patch and directed toward the mouth of the great vessels which take origin from the aortic arch. In some instances this latter plaque may be slightly separated from the sclerotic patch, but yet in an approximate line drawn through its long axis. In



Fig. 7. Macroscopic appearance of typical plaque at site of aortic insertion of ligamentum arteriosum.

other instances it fuses with others at the mouth of the large vessels. There are, of course, numerous variations.

The calcification of the aortic knob visualized on the clinical x-ray film presents as the outline of the vessel on posterior-anterior examination. At times only the lower segment of the circle is seen. In other instances the lower, lateral and upper segments are noted and less frequently the entire circumference. The medial portion is visualized with least frequency. This can be explained in part by the distribution of arteriosclerotic alterations in the aortic knob, but there are also shadows of contiguous structures which may obscure the aorta.

The sclerotic patch and the contiguous areas of sclerosis in the aortic isthmus are so situated during life that in an anterior-posterior projection they are superimposed on one another to form a ring-like structure (Figs. 3 and 4). The anterior-posterior x-ray films penetrate various portions of the thickness of this ring. It follows that this projection is the technically desirable one to demonstrate calcification of the aortic knob. This may easily be corroborated

by lateral and oblique x-ray films of patients who show a well defined plaque in the posterior-anterior projections.⁵ In positions other than posterior-anterior the calcification within the knob is seen less clearly or not at all.

From the findings in this study it is evident that the radiographically visualized calcification of the aortic knob is a local phenomenon and represents the site of sclerosis in the vicinity of the aortic insertion of the obliterated ductus botalli and along the aortic isthmus. Gubner⁶ infers that calcification of the aortic knob may be an indicator of arteriosclerosis. The findings of this study are contrary to this impression. Hyman and Epstein⁷ state that radiographically visualized aortic calcification is indicative of severe arteriosclerotic changes. The findings in the present study are in agreement with this statement. It must, however, be stressed that this pertains only for the site visualized and that specifically the calcification of the aortic knob is not an indicator of the extent or severity of the lesions in the remainder of the aorta.

SUMMARY

By means of postmortem examination of the aorta, including postmortem roentgenogram, and correlation with antemortem x-ray films, it is shown that the linear calcification visualized in the aortic knob is in almost all instances a representation of arteriosclerotic alterations in the vicinity of the aortic insertion of the ligamentum arteriosum, along the aortic isthmus and at the origins of the large vessels.

This alteration is a local phenomenon and has no relation to the degree of arteriosclerosis in the remainder of the aorta.

The calcification seen in the lowest sector of the circumference of the aortic knob almost always indicates the site of aortic insertion of the ligamentum arteriosum and the linear calcification seen on the upper margins most frequently delineates a plaque at the mouth of the large vessels taking origin from the arch.

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Seminar on The Quantitative Aspects of Atherosclerosis

Introduction

THE most common serious clinical sequel of atherosclerosis is overt coronary heart disease. The past ten years has seen the evolution of a strong body of evidence linking certain blood lipids with the development of the clinical entity of coronary heart disease. While controversy has existed concerning the relative merits of several types of blood lipid measurements, few serious investigators today doubt the blood lipid-coronary heart disease relationship. Thus, the clinician has one solidly established factor to consider in evaluating a person's potential for development of coronary heart disease. Despite this the astute clinician knows that certain problems remain. For example, there is the occasional patient with fatal coronary heart disease in whom autopsy study reveals one branch, or part of a branch, of a coronary artery severely involved with atherosclerosis while other regions of the coronary tree are relatively free of this disease. Further, some of these patients may have been characterized by modest levels of atherosclerosis-associated blood lipids. None of these facts in any way invalidates the strong relationship between blood lipid levels and clinical consequences of coronary atherosclerosis. Rather, they point up the crucial fact, realized early, that over and above general metabolic factors related to atherosclerosis, there must exist highly important determinative factors in the arterial wall itself or in the relationship of blood flow to the arterial wall.

Our ultimate clinical objective clearly is the earliest development of an ability to prevent and treat effectively clinical consequences of atherosclerosis, in the coronary, the cerebral and the peripheral arterial beds. Recognition of such general factors as blood lipid levels is one important step in this direction. There can be no doubt that a clearer understanding of the mechanism of operation of local factors will represent a second step of consequence. This seminar is directed toward statement of the problem of such local arterial factors and the light shed upon this problem by some recent quantitative investigations.

JOHN W. GOFMAN, M.D., F.A.G.C.

Donner Laboratory

University of California

Berkeley, California

The Quantitation of Atherosclerosis

I. Relationship to Artery Size*

Wei Young, ph.d., John W. Gofman, m.d., f.a.c.c., Robert Tandy,

Berkeley, California

NATHAN MALAMUD, M.D. and EUNICE S. G. WATERS, M.D.

San Francisco, California

Napa, California

I'N the past three decades the evaluation of atherosclerosis has been in a gradual transition from purely qualitative and intuitional approaches to highly quantitative direct measurements. Early attempts at quantitation of atherosclerosis in the cerebral arteries were made by Volkoff¹ in 1933. More recent studies directed toward quantitation are exemplified by the broad evaluation of coronary atherosclerosis by White, Edwards and Dry.2 In all these studies the severity of atherosclerosis was graded roughly from 0 to 4. The roughness of grading in previous studies provides data inadequate to understand the intricate interrelationship between atherosclerosis and other parameters which are highly quantitatively measured. Thus, in the past, many probable relationships have been completely obscured.

It is generally agreed that atherosclerosis is more prevalent in the large- and medium-sized arteries than in the arterioles. However, the degree of atherosclerosis specifically as a function of the size of an artery has never been studied quantitatively. Without measuring arterial size, White and his associates2 demonstrated that the degree of atherosclerosis was much higher in the proximal part of a particular coronary artery than in the distal part. This observation suggests the possibility of some relationship between the distribution of atherosclerosis and the size of the coronary artery, since the proximal part of an artery is usually wider than its distal part. Subsequently, Blumenthal,8 after combining his observations on atherosclerosis with the data on dogs of Green⁴ and the calculations of Burton, ⁵ also

proposed that the incidence of plaque formation was higher in arteries with large lumens than in those with small lumens. However, his grading measurements indicated a somewhat lower degree of atherosclerosis in the internal carotid artery than in the middle cerebral and basilar arteries. Winter and his associates also noted this apparent discrepancy in the distribution of cerebral atherosclerosis by their methods of grading. These discrepancies could certainly arise from inconsistencies in the technics used for grading. With a truly quantitative measure of atherosclerosis this problem may be clarified.

Analysis of the degree of atherosclerosis along any branch of an artery can also serve to differentiate the contribution of local factors from that of general metabolic ones⁷⁻⁹ in the atherosclerotic process. For such analyses it is essential to have some definite quantitative measure of atherosclerosis as well as of the size of the artery. This report presents a technic for the quantitation of the extent of atherosclerosis in any artery and the results obtained from application of this technic to human tissue samples.

In the past several years we have applied this technic principally to the coronary and cerebral arteries along their major branches.¹⁰ This report is primarily concerned with the interrelationship between the size of an artery and the degree of its atherosclerosis. The relationship of calcification to distance is also considered.

MATERIAL AND METHODS

This study is based upon 4,331 sections of coronary

^{*} From the Division of Medical Physics, Donner Laboratory of Medical Physics, Radiation Laboratory, University of California, Berkeley, California; The Langley Porter Clinic, San Francisco, California; and The Napa State Hospital, Napa, California.

Fig. 1. Segments of the arteries analyzed for atherosclerosis. Upper, sixteen segments of coronary arteries. Lower, twenty-four segments of brain arteries.

and cerebral arteries removed at autopsy (in the Napa State Hospital) from 143 consecutive hearts and brains. Sixteen standard sections were taken from the heart and twenty-four sections were taken from the brain (Figs. 1A and 1B). Histologic sections were prepared by standard technics and stained with hematoxylin and eosin. Sections were occasionally stained according to the method of hematoxylin Van Gieson, counterstained with that of Weigert to differentiate the amount of collagen and elastic tissue in different layers.

The quantitation of the atherosclerotic process in any arterial section was based on the direct measurement of the physical changes in the arterial tissue. The most obvious physical change concomitant with the atherosclerotic process is the increase in the intimal material. Since the amount of intimal tissue is negligible in the uninvolved artery, for a cross section of artery the total area of intimal tissue (all tissue between the endothelial surface and the internal elastic lamella) was considered as evidence of accumulation of atherosclerosis. In order to correct for variation in the size of artery from section to section and from case to case the area of intimal atherosclerotic material was referred to the total arterial cross sectional area of each section and the

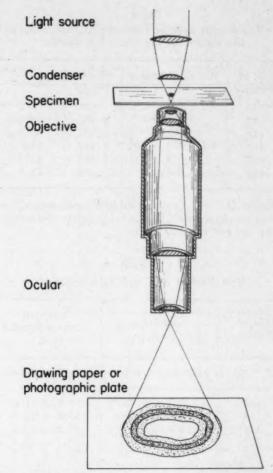


Fig. 2. Schematic representation of assembly for enlargement of histological arterial section.

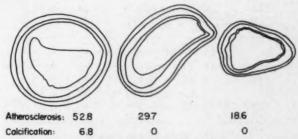


Fig. 3. Typical tracings and planimetric measurements.

results expressed as a percentage of the total cross section. Atherosclerosis will thus be defined in this study by the following:

$$A the rosclerosis = \frac{I(area\ of\ intimal\ material)}{E(total\ arterial\ cross-section\ area)}$$

All area measurements were made by planimetry on a precisely enlarged tracing of the histological arterial section. The degree of calcification was derived from the calcified area in the histological slide by similar area measurement. No attempt was made to grade the early phase of calcification. How-

TABLE I

The Relationship Between Coronary Atherosclerosis and the Radius of the Coronary Arteries

No. of Sections	Range of Radius (mm.)	Mean r (mm.)	(I/E)c
48	2.6-3.0	2.672	58.2
152	2.1-2.5	2.206	58.5
212	1.6-2.0	1.737	55.0
228	1.0-1.5	1.253	43.8
118	0.5-1.0	0.798	28.1

Note: (I/E)c = Coronary atherosclerosis, where, I = intima material area; E = total arterial cross section area; and c = coronary.

TABLE II
The Arterial Size and Atherosclerosis

No. of Sections	Radius (mm.)	Degree of Atherosclerosis (I/E)
A. Anterio	r Descending Branch of L	eft Coronary Artery
135	2.097 ± 0.350*	59.8 ± 11.8
139	1.650 ± 0.370	54.8 ± 13.7
139	1.376 ± 0.344	48.5 ± 15.4
125	1.200 ± 0.307	42.7 ± 16.4
105	1.036 ± 0.318	32.9 ± 14.3
	B. Left Anterior Bro	anch
135	1.131 ± 0.392	38.9 ± 18.3
123	0.981 ± 0.319	34.7 ± 15.1
99	0.839 ± 0.279	28.0 ± 13.8
	C. Lest Circumstex B	ranch
139	1.864 ± 0.439	55.1 ± 13.4
134	1.636 ± 0.416	50.2 ± 13.5
123	1.380 ± 0.399	43.4 ± 16.1
	D. Right Coronary A	rtery
143	2.066 ± 0.411	56.6 ± 10.5
144	1.883 ± 0.387	53.5 ± 11.6
128	1.763 ± 0.458	50.1 ± 13.8
101	1.607 ± 0.458	46.7 ± 14.5

^{*} Standard deviation of distribution.

ever, it could be graded by counting the calcified granules under higher magnification.

Figure 2 shows the tracing arrangement, which is essentially a projecting microscope. A photographic plate can also be used instead of an enlarged tracing. Three typical tracings are illustrated in Figure 3.

TABLE III

The Relationship Between Cerebral Atherosclerosis and Radius of Cerebral Arteries

No. of Sections	Range of Radius (mm.)	Mean Radius (mm.)	(I/E)b
37	2.1-2.5	2.246	58.5
151	1.6-2.0	1.700	49.0
518	1.1-1.5	1.214	32.0
354	0.6-1.0	0.867	19.8
2	0.2-0.5	0.470	18.0

Note: (I/E)b = Atherosclerosis of the brain, where I = intimal material area; E = total arterial cross-section area; and b = brain.

The degree of atherosclerosis and of calcification, is indicated respectively, at the bottom of each tracing.

RESULTS

All sections of coronary and cerebral arteries were grouped into nine categories (0 to 0.50, 0.51 to 0.75, 0.76 to 1.00, 1.01 to 1.25, 1.26 to 1.50, 1.51 to 1.75, 1.76 to 2.00, 2.01 to 2.50 and 2.51 to 3.00 mm. in radius) according to their size, disregarding the arterial branch they represent. The distribution of coronary and cerebral arterial sections are listed in Tables 1 and 111 separately.

Atherosclerosis and the Radius of Coronary Arteries: The relationship of atherosclerosis to the radius of the coronary artery is shown in Table 1. The degree of atherosclerosis is significantly related to the radius of the artery.

These data show that the bigger the artery, the higher the degree of atherosclerosis. However, for radii above 2 mm. there appears a plateau since further increases in the radius of the artery are not associated with proportional increases in atherosclerosis values.

An analysis of the relationship between atherosclerosis and the radii of the different arterial branches is shown in Table II. All individual branches of the coronary arteries show a similar trend; that is, the degree of atherosclerosis decreases toward the peripheral end of the artery. The anterior descending branch shows the highest sclerosis (I/E = 59.8) in the proximal part, yet it decreases sharply at its distal end (I/E = 33). The left circumflex and the right coronary artery yield I/E values of 55 and 56 for the proximal part and 43 and 46 for the distal part. This discrepancy in degree of atherosclerosis in the distal parts of these dif-

TABLE IV
The Cerebral Arterial Size and Atherosclerosis

No. of Sections	Left Radius	Degree of Atherosclerosis	No. of Sections	Right Radius	Degree of Atherosclerosis (I/E
		A. Posterio	Cerebral Arteri	ies	
143	1.213 ± 0.273	33.4 ± 19.4*	139	1.22 ± 0.303	34.5 ± 20.5*
135	1.053 ± 0.243	29.5 ± 19.5	135	1.046 ± 0.285	31.0 ± 20.1
114	0.888 ± 0.260	26.5 ± 16.8	105	0.893 ± 0.279	27.0 ± 16.7
		B. Middle	Cerebral Arterio	es	
138†	1.767 ± 0.403	39.2 ± 19.5	124	1.800 ± 0.423	39.4 ± 18.3
140	1.466 ± 0.307	37.3 ± 20.6	140	1.451 ± 0.294	37.1 ± 20.5
137	1.178 ± 0.260	31.8 ± 20.4	134	1.209 ± 0.291	33.0 ± 19.8
121	0.965 ± 0.239	25.7 ± 16.5	119	1.000 ± 0.248	26.9 ± 17.8
		C. Anterior C	erebral Arteries		
138	1.144 ± 0.254	25.7 ± 15.4	140	1.170 ± 0.261	27.3 ± 16.6
133	0.988 ± 0.230	23.5 ± 15.0	137	1.090 ± 0.238	24.0 ± 15.1
110	0.867 ± 0.192	22.8 ± 13.6	108	0.867 ± 0.204	23.3 ± 13.8

* Standard deviation of distribution.

† Internal carotid artery.

ferent branches can be easily explained by the fact that the actual sizes are different in these branches (Table IIA, C and D).

Atherosclerosis and the Radius of Cerebral Arteries: A similar distribution of the degree of atherosclerosis was found in all branches of the cerebral arteries so far studied (Table III). The highest degree of sclerosis was found in the middle cerebral arteries. The degree of sclerosis in the posterior cerebral arteries was less than in the middle cerebral arteries. The least severe was in the anterior cerebral arteries (Table IVA, B and C).

There was practically no difference between the atherosclerosis of the cerebral arteries of the left side and that on the right side. This was probably due to the almost symmetrical anatomical distribution of the cerebral arteries.

The degree of atherosclerosis of the internal carotid artery was found to be higher (I/E = 39) than that of the middle cerebral artery (I/E = 37, 32, 26) but was definitely lower than the highest value in the basilar artery (I/E = 42, 38, 31).

Interrelationship Between Atherosclerosis and the Size of Arteries: The data for the over-all relationship between atherosclerosis and the radius of the artery, including all coronary and

cerebral vessels studied, are shown in Figure 4. There is almost a linear relationship between the degree of atherosclerosis and the radius of the artery. The Pearson coefficient of correla-

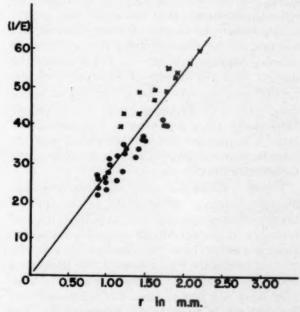


Fig. 4. Relationship between atherosclerosis (I/E) and radius (r) of the artery. x, coronary arteries, \bullet , cerebral arteries. Each point represents an average of ≥ 100 sections.

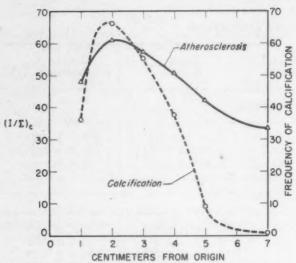


Fig. 5. Atherosclerosis and calcification versus distance from the origin of anterior descending branching of left coronary artery.

tion (r) is 0.77 when calculated from the data derived from 4,331 artery sections. The p value is much lower than 0.001 and the relationship between atherosclerosis and radius is therefore highly significant. The atherosclerotic gradient was found to be in the neighborhood of 2.14 (I/E) units per 100 μ change in the radius of the artery.

Atherosclerosis and the Distance from the Origin of the Arterial Branch: Another approach to a study of the relationship between the size of the artery and the degree of sclerosis (but independent of measuring the radius of the artery) is to measure the degree of atherosclerosis as a function of the distance from the origin of the arterial branch. White and his associates² showed that the degree of atherosclerosis decreased progressively from the proximal to the distal part of the coronary artery in any decade from thirty to ninety years. It would be of interest to measure the actual distance and the exact amount of intimal mass according to the technic described herein.

Figure 5 shows that relationship between the distance from the origin of the artery and the degree of sclerosis based on 1,670 sections of coronary arteries. All the branches measured show a similar trend of sclerosis, that is, the atherosclerosis decreases toward the peripheral ends. In the anterior descending branch, however there is a higher degree of sclerosis 2 cm. from its origin than at the origin itself. The slightly higher value at 6 cm. from the origin for the right coronary artery (in comparison with the anterior descending branch) can be

explained if the actual radius is taken into account. The radius of the right coronary at 6 cm. is comparable with the radius at 4 cm. for the anterior descending branch in most cases.

Calcification of Arteries and the Distance from the Origin of the Arterial Branch: Figure 5 also shows, for the anterior descending branch, the relationship between the frequency of calcification* and the distance of the arterial section from its origin. The highest incidence of calcification is 2 cm. from the origin. The calcification approaches 0 at 6 or 7 cm. from the origin. The cerebral arteries are nearly free from calcification.

COMMENTS

The quantitative studies reported here establish a significant relationship between atherosclerosis and (1) size of artery and (2) distance from the origin of artery. From these data an atherosclerosis gradient can be evaluated, which can be shown to have definite physical interpretation in terms of area measurements.

Factors responsible for such a gradient may be listed as follows: (1) general metabolic factors such as lipoproteins, hormones and heparin and (2) local factors such as pressure and turbu-Atherosclerosis and lipoproteins have been studied extensively.7-9 Furthermore, it has been found that certain species of lipoproteins are especially associated with the occurrence of atherogenesis.11 Since these measurements were carried out in the same branch of the arterial system, the influence of a general metabolic factor such as lipoproteins may be considered to be effectively the same throughout an individual branch. Thus, attention is directed to local factors such as pressure and turbulence in determining the nature of this atherosclerosis gradient.

If the pressure gradient is calculated† along any arterial branch and the resulting gradient compared with the distribution of quantitative atherosclerosis there is some similarity in the distribution of degree of atherosclerosis and pressure.⁴ Such comparison will be reported elsewhere in detail.¹² Notwithstanding the

^{*} Frequency of calcification = number of arterial sections showing calcified area of 5u² or more/number of arterial sections studied.

[†] Pressure drop = $f(1/d)(V^2/2g)(P1/P2)$, where f = friction factor; l = length of the artery; d = diameter of the artery; V = velocity of blood flow; g = gravity acceleration; P_1 = specific gravity of blood; P_2 = specific gravity of Hg.

difficulties in explaining the exact cause of the observed atherosclerosis gradient it is now clear that such a gradient exists and can be related to such important factors as intravascular pressure.

The measurement of atherosclerosis at different distances from the origin of an arterial branch not only confirms but also extends the observations made by White, Edwards and Dry.² The frequency and severity of calcification also appear to be related similarly to the distance (Fig. 5). It is worthwhile to note that the calcification in cerebral arteries is much less frequent and less severe than that in the coronary arteries.

Examination upon dissection of the arteries frequently shows plaques to be scattered all the way along an arterial branch. An artery of considerable length uniformly and concentrically coated with lipid material without localized regions of atherosclerosis is quite rare. On the contrary, in the majority of cases plaques are deposited eccentrically. Unfortunately, our measurements in this study did not differentiate between concentric or eccentric deposits. Nonetheless, attention can be directed to certain strategic spots as in section 2 (Fig. 1A) of the anterior descending branch of the left coronary artery which is particularly prone to deposition. This section shows the highest deposition among all the branches measured despite an average smaller radius than section 1. The eccentric deposition and the geometrical distribution of the plaque cannot be explained readily by the pressure gradient alone. It seems more likely that other hemodynamic factors play a major role in such arterial plaque formation. The influence of turbulent flow and shear stress on the arterial wall with respect to plaque formation will be discussed elsewhere.12

SUMMARY

- 1. A quantitative method for grading atherosclerosis in arteries is described.
- 2. The radius of the artery and the degree of atherosclerosis in 4,331 sections of coronary and cerebral arterial sections were quantitatively measured by this method.

- 3. The degree of atherosclerosis was found to be highly correlated with the radius of the artery. The arteries with larger radii showed a significantly higher degree of sclerosis.
- 4. The severity of sclerosis was also correlated with the distance of sections from the origin of any branch of the artery.
- 5. The distribution and properties of an atherosclerosis gradient and an intravascular pressure gradient are discussed.

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The Quantitation of Atherosclerosis

II. Quantitative Aspects of the Relationship of Blood Pressure and Atherosclerosis*

WEI YOUNG, PH.D., JOHN W. GOFMAN, M.D., F.A.C.C., ROBERT TANDY, Berkeley, California

NATHAN MALAMUD, M.D. and EUNICE S. G. WATERS, M.D. San Francisco, California

Napa, California

THE blood pressure as a physiologic variable L has been of great interest both in respect to its relationship with atherosclerosis itself and with such clinical consequences of atherosclerosis as coronary heart disease or cerebrovascular disease. The extent to which hypertension is related to the probability of development of clinical coronary heart disease has been recently evaluated in quantitative terms,1 based upon follow-up studies^{2,8} linking pressure to such disease. Clinically, it is almost accepted as an axiom that hypertension is closely related to certain forms, at least, of cerebrovascular disease. Such clinical relationships can imply either that hypertension derives such relationship via its effect upon the underlying atherosclerotic process or via its effect upon some later event in the chain leading to clinical sequelae. Experimental evidence both in the dog4 and rabbit⁸ points strongly to an effect of hypertension in acceleration of the actual development of atherosclerosis itself.

Davis and Klainer⁶ reported on the incidence of coronary sclerosis, measured semiquantitatively, in persons with and without hypertension. They concluded that subjects with hypertension showed more coronary sclerosis than those without hypertension, and that this difference was more marked in the earlier decades of life than in the late decades of life. However, they also concluded that the hypertension per se did not appear to be the prime factor in determination of degree of coronary sclerosis.

In this discussion data are presented concerning the quantitative relationship between systolic and diastolic blood pressures and the degree of atherosclerosis in two crucial vascular beds, namely, the coronary arteries and the cerebral arteries. These data on man, in agreement with those for the dog and the rabbit, indicate that antecedent hypertension is truly related to the development of atherosclerosis in the coronary and cerebral arterial beds, and that in all likelihood it is this relationship that accounts for part or all of the observed relationship between antecedent hypertension and the subsequent increased risk of clinical coronary or cerebral vascular disease.

MATERIAL AND METHODS

Ideally, it would be helpful to have records of the systolic and diastolic blood pressures measured serially over the lifetime of a group of persons and then to measure quantitatively the degree of coronary and cerebral atherosclerosis at autopsy. Unfortunately, such data are unavailable at this time. As a first step toward this ideal goal, it was considered worthwhile to have at least some measurement of systolic and diastolic blood pressure during life for a group of subjects in whom coronary and cerebral vessels were to be available for study at autopsy. The same group of subjects described in the first section of this seminar7 represents the potential substrate for such a study. Records of at least one measurement of systolic and diastolic blood pressure are available for ninety-five of 143 such subjects. No pressure readings were available for the remaining forty-eight subjects in whom the degree of sclerosis had been quantitatively evaluated. Although this reduces the size of the

^{*} From the Division of Medical Physics, Donner Laboratory of Medical Physics, Radiation Laboratory, University of California, Berkeley, California; The Langley Porter Clinic, San Francisco, California; and The Napa State Hospital, Napa, California. This work was supported by the Albert and Mary Lasker Foundation and the Samuel Roberts Noble Foundation and the U. S. Atomic Energy Commission.

TABLE 1 Cerebral Atherosclerosis and Blood Pressure

Age (yr.)	Cases (no.)	Degree of Atherosclerosis	Blood Pressure (mm. Hg)	Pearson Coefficient of Correlation	Significance Test
		Anterio	or Cerebral Artery		
60-69	19	16.3 ± 12.4*	S 156 ± 33†	0.70	<0.001
70-79	49	15.1 ± 11.3	D 91 ± 17 S 159 ± 35	0.61 0.17	<0.01 N.S.
70-79	47	15.1 ± 11.5	D 89 ± 15	0.11	N.S.
80-89	27	13.5 ± 7.1	S 151 ± 13	0.39	< 0.05
			D 82 ± 15	0.57	<0.01
60-89	95	14.9 ± 10.6	S 156 ± 31 D 87 ± 15	0.35 0.35	<0.01 <0.01
		Middl	e Cerebral Artery		
42 42 1		1		0.77	-0.004
60-69	19	19.9 ± 14.4	S 156 ± 33 D 91 ± 17	0.67	<0.001 ~0.001
70-79	49	19.9 ± 13.4	S 159 ± 35	0.32	<0.05
10-12	. 42	17.7 = 15.4	D 89 ± 15	0.32	< 0.05
80-89	27	20.5 ± 13.2	S 151 ± 13	0.32	N.S.
			D 82 ± 15	0.64	< 0.001
60-89	95	20.1 ± 13.6	S 156 ± 31	0.40	< 0.001
	-		D 87 ± 15	0.49	<0.001
		Posterie	or Cerebral Artery		
60-69	.19	19.2 ± 14.6	S 156 ± 33	0.67	< 0.001
	40	400.400	D 91 ± 17	0.67	< 0.001
70-79	49	19.8 ± 12.9	S 159 ± 35 D 89 ± 15	0.26 0.28	N.S. ~0.05
80-89	27	21.5 ± 13.8	S 151 ± 13	0.50	< 0.01
00 0,		21.5 = 15.0	D 82 ± 15	0.65	< 0.001
60-89	95	20.2 ± 13.6	S 156 ± 31	0.40	< 0.001
			D 87 ± 15	0.47	<0.001
		Basilar a	and Carotid Arteries		
60-69	19	22.9 ± 14.5	S 156 ± 33	0.66	< 0.001
	* -	45. 4	D 91 ± 17	0.71	< 0.001
70-79	49	23.4 ± 12.7	S 159 ± 35	0.36	< 0.01
20 00	27	21.5 ± 13.8	D 89 ± 15 S 151 ± 13	0.39	<0.05 N.S.
80-89	21	21.5 ± 13.8	D 82 ± 15	0.50	<0.01
60-89	95	22.8 ± 12.6	S 156 ± 31	0.44	<0.001
00-89	73	22.6 ± 12.0	D 87 ± 15	0.48	<0.001
		Average Cerebral Ath	erosclerosis and Blood Pr	ressure	
60-69	19	19.4 ± 13.4	S 156 ± 33	0.71	< 0.001
			D 91 ± 17	0.69	< 0.001
70-79	49	19.5 ± 11.6	S 159 ± 35	0.32	< 0.05
00.00	25	40.0 . 40.4	D 89 ± 15	0.30	~0.05
80-89	27	19.2 ± 10.4	S 151 ± 13	0.40	< 0.05
60.00	05	10 4 2 11 7	D 82 ± 15 S 156 ± 31	0.66	<0.001
60-89	95	19.4 ± 11.7	S 156 ± 31 D 87 ± 15	0.44	< 0.001

* All values of degree of atherosclerosis are given in units of I/E (see preceding part of this seminar⁷) together with the standard deviation of the distribution of the values.

† Pressures (S = systolic; D = diastolic) recorded in the arm. The values are given together with the standard deviation of the distribution of values.

TABLE II
Coronary Atherosclerosis and Blood Pressure

Age (yr.)	Cases (no.)	Degree of Atherosclerosis	Blood Pressure (mm. Hg)	Pearson Coefficient of Correlation	Significance Test*
		Lej	ft Main Artery		
60-69	19	39.1 ± 10.5	S 156 ± 33 D 91 ± 17	0.73 0.61	<0.001 <0.01
70-79	49	32.9 ± 13.3	S 159 ± 35 D 89 ± 15	0.06 -0.08	N.S. N.S.
80-89	27	34.2 ± 12.9	S 151 ± 23 D 82 ± 15	-0.10 -0.19	N.S. N.S.
60-89	95	34.5 ± 12.9	S 156 ± 31 D 87 ± 15	+0.13 0.14	N.S. N.S.
		Anterior	Descending Branch		
60-69	19	32.5 ± 14.5	S 156 ± 33 D 88 ± 17	0.38	N.S. N.S.
70-79	49	37.6 ± 11.1	S 159 ± 35 D 89 ± 15	-0.22 -0.07	N.S. N.S.
80-89	27	37.0 ± 11.8	$\begin{array}{ccc} S & 151 \pm 23 \\ D & 82 \pm 15 \end{array}$	0.16 0.40	N.S. <0.05
60-89	95	36.4 ± 12.2	S 156 ± 31 D 87 ± 15	0.01 0.12	N.S. N.S.
		Left	Anterior Branch		
60-69	19	28.3 ± 18.8	S 156 ± 33 D 91 ± 17	0.05	N.S. N.S.
70-79	49	23.3 ± 10.4	S 159 ± 35 D 89 ± 15	-0.01 0.14	N.S. N.S.
80-89	27	29.2 ± 12.9	S 151 ± 23 D 82 ± 15	-0.18 0.20	N.S. N.S.
60-89	95	26.0 ± 13.4	S 156 ± 31 D 87 ± 15	0.05 0.24	N.S. <0.05

series available for study of the relationship of blood pressure to the degree of sclerosis, it is difficult to see how any conceivable bias is thereby introduced. Since the degree of sclerosis was not known during life, this can hardly have been a factor in determining whether or not pressure measurements were available. The data presented subsequently therefore represent the material and evaluation thereof in assessment of the blood pressure-atherosclerosis relationship. Quantitative measurement of the degree of atherosclerosis was made in the manner outlined in detail previously⁷ and the degree of sclerosis is reported in units of I/E.

RESULTS

The measurements of blood pressures and the degree of cerebral atherosclerosis are presented in Table 1.

The measurements of blood pressure and

coronary atherosclerosis are presented in Table II.

CEREBRAL ATHEROSCLEROSIS AND BLOOD PRESSURE

The data in Table 1 show that for all regions of the cerebral arterial bed considered (including basilar, carotid, anterior, middle and posterior cerebral arteries) there is a trend toward an increase in the degree of atherosclerosis with an increase in systolic blood pressure and a similar trend for cerebral atherosclerosis with diastolic pressure. For all vessels studied the positive correlation coefficient observed can be proved significant for the over-all series of patients over the age range from sixty to eighty-nine years. For all vessels the observed correlation is strongest for the sixty- to sixty-nine-year age group. For certain subsegments of the over-all

TABLE II (Continued)
Coronary Atherosclerosis and Blood Pressure

Pearson Efficient of Signification Signification Signification		Blood Pressure (mm. Hg	(Degree of Atherosclerosis	Cases (no.)	Age (yr.)
		Aex Branch	ft Circumfl	Left		
0.21 N.S.		156 ±	s	42.7	19	60-69
0.15 NS.		91 ±	D			
0.12 N.S.		159 ±	S	38.3	49	70-79
0.10 N.S. 0.02 N.S.		89 ± 151 ±	D	36.8	27	80-89
0.02 N.S. 0.30 N.S.		82 ±	D	30.8	21	80-89
0.13 N.S.		156 ±	S	38.8	95	60-89
0.20 ~0.05			D	30.0	-	00 07
10/2		ary Artery	ight Corona	Righ		
0.68 <0.00	3	156 ±	S	39.3 ± 9.4	19	60-69
0.44 <0.05		91 ±	D		1	
0.19 N.S.		159 ±	S	43.1 ± 10.0	49	70-79
0.12 N.S.		89 ±	D	20 0 1 40 0	07	00 00
0.04 0.09 N.S. N.S.		151 ± 82 ±	S	38.0 ± 12.0	27	80-80
0.26 <0.05		156 ±	S	40.9 ± 10.8	95	60-89
0.20 ~0.05		87 ±	D	40.7 2 10.0	23	00 07
	lood Pressu	erosis and	Atheroscles	Average Coronary A	,	
0.56 <0.01	3	156 ±	S	37.3 ± 9.5	19	60-69
0.42 ~0.05	7	91 ±	D			
0.09 N.S		159 ±	S	36.9 ± 7.5	49	70-79
0.11 N.S.		89 ±	D			
0.01 NS.		151 ±	S	36.3 ± 8.6	27	80-89
0.33 N.S.		82 ±	D			
				36.8 ± 8.2	95	60-89
0.19 ~	1	156 ± 87 ±	S	36.8 ± 8.2	95	60-89

^{*} N S = not significant

group of cases, the correlation coefficients, while positive, are not large enough for the number of cases studied to be independently at the 5 per cent level of significance. We may regard the observed correlation coefficients to be quite high when considered in the light of the circumstances necessary under the available conditions for study. If a variable such as blood pressure relates to the rate of development of cerebral atherosclerosis, the best assessment would require a knowledge of serial blood pressure readings over the entire interval during which atherosclerosis had been developing. Since such readings were not available, we are essentially forced to use the single elevated pressure reading as an indication of the probability that multiple readings would have revealed a tendency toward elevated pressures in the particular subject. This is admittedly far from ideal. However, it can be said that the relatively high correlation coefficients observed here between blood pressures based upon a single evaluation during life and the degree of atherosclerosis probably infers that the true relationship between blood pressure levels averaged over a long period of years and degree of cerebral sclerosis would even be appreciably higher.

CORONARY ATHEROSCLEROSIS AND BLOOD PRESSURE

The data in Table II indicate that within the

over-all group of cases for the average of all regions of the coronary arterial tree, the relationship between blood pressure, systolic or diastolic, and the degree of atherosclerosis, is much less prominent than is the relationship between cerebral atherosclerosis and blood pressure. As is the case for cerebral atherosclerosis, the trend observed is for the highest relationship between blood pressure and coronary sclerosis to be observed in the sixty- to sixty-nine-year age group (the youngest subsegment of the over-all group studied). Further, it is observed (both for systolic and diastolic pressure) that the strength of the relationship between pressure and degree of sclerosis is greater for the main coronary branches (left and right) than for the remainder of the coronary arterial tree. Again, as already stated for the cerebral arterial case, any observed relationship between the degree of sclerosis and blood pressure is probably less than would have been measured were much more extensive blood pressure records available.

COMMENTS

Three interesting questions present themselves for consideration: (1) Why should the relationship between cerebral atherosclerosis and blood pressure be apparently so much stronger than that between coronary sclerosis and blood pressure? (2) Why should the relationship between atherosclerosis of main coronary branches with blood pressure be appreciably greater than that observed between more distal coronary branches and the blood pressure? (3) Why should the extent of relationship between blood pressure and degree of atherosclerosis in any arterial bed be less with increasing age (in the range of sixty to eighty-nine years)? Two issues of importance may help clarify in part the answers to these questions.

First, if it be considered that blood pressure comes to be related to degree of atherosclerosis as a hydrodynamic factor, it would follow that the real pressure desired for such a study would be the pressure actually operative in the particular arterial branch under consideration. But the pressures measured in this study are arm blood pressures. Hence, with respect to such hydrodynamic considerations, the extent of relationship of pressure with degree of atherosclerosis would depend upon the extent to which arm blood pressure reflects pressure in the artery branch under study. It seems reasonable that the arm blood pressure and that

existing in the major cerebral branches would be more closely related than would the arm blood pressure and that existing in the coronary branches, simply because of the disturbing effect of the pumping and twisting action of the heart upon this relationship. Furthermore the distortion would be less for the relationship between main coronary branch pressures and arm blood pressure than for the relationship between more distal coronary branch pressures and arm blood pressure. If such considerations are valid, then a partial answer would be available for the first two questions raised.

A second factor also deserves consideration. This relates to the effect of existing degree of atherosclerosis in a particular artery upon the subsequent development of new, or additional, sclerosis. In the preceding communication of this seminar7 it was shown that atherosclerosis decreases as the radius of the artery decreases. When an appreciable degree of atherosclerosis has developed in a vessel, the inner radius of that vessel must necessarily have decreased, unless the vessel has undergone over-all dilatation. Reference to Tables 1 and 11 shows that at any particular age the degree of coronary sclerosis is much greater than the degree of cerebral sclerosis. Therefore, the effective inner radius of the coronary vessels would become less as the degree of sclerosis increased, unless dilatation of significant degree had occurred. By separate test of the data it is possible to show, within any of the age groups considered, or for the over-all group, that the outer diameter of a particular coronary artery is not significantly changed as the degree of atherosclerosis increases. In other words measurable dilatation (at least for autopsy material) does not accompany development of atherosclerosis. This means, necessarily, that as the degree of atherosclerosis rises the inner radius of the vessel decreases. This should, therefore, imply that new or additional sclerosis should develop more slowly, all other factors being equal, simply because of the radius effect. This helps explain the difference in correlation of blood pressure with cerebral sclerosis and blood pressure with coronary sclerosis. In the cerebral vessels, total development of sclerosis is much less than in coronary vessels, at a particular age, and hence the inner radius of the cerebral vessel in which sclerosis is developing remains nearly constant. In contrast, as the appreciable sclerosis characteristic of the coronary arteries develops, appreciable radius reduction is occurring and hence new sclerosis is always developing in a vessel of progressively smaller radius. Thus a relationship such as that of blood pressure to the degree of sclerosis is much more disturbed by the radius effect in the coronary vessels than in the cerebral vessels.

Neither the explanation of the extent to which arm blood pressures reflect particular coronary or cerebral vessel pressure nor the explanation based upon radius decrease with increasing sclerosis helps answer the third question raisednamely, why all the correlations observed between pressure and degree of sclerosis fall off in the older age groups. No good explanation can be offered for this effect. One speculation would be that the pressure measured in the seventy- to eighty-nine-year age group reflects less well the habitual pressure that characterized the period of development of sclerosis than does the measured pressure in the sixty- to sixty-nine-year age group. This is, of course, one facet of the non-ideal character of material for such a study based upon a single measurement of the blood pressure during life.

SUMMARY

1. Systolic and diastolic pressures are positively and significantly correlated with the degree of coronary sclerosis and the degree of cerebral sclerosis.

2. The strength of the relationship of blood pressure to the degree of atherosclerosis is much greater for the cerebral arterial bed than for the coronary arterial bed.

3. The strength of the relationship of blood pressure to the degree of atherosclerosis is greater for proximal coronary artery branches than for distal branches.

4. The strength of the correlations observed both for cerebral and coronary vessels with blood pressure are less for subjects in the seventy-to eighty-nine-year age group than for those in the sixty- to sixty-nine-year age group.

5. Reasonable explanations can be proposed to explain, at least in part, the observations of (2) and (3), based upon known physiologic data or other information concerning quantitative atherogenesis. The absence of serial measurements of blood pressure precludes the testing of certain speculations to explain the change in correlation strength with age.

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The Quantitation of Atherosclerosis

III. The Extent of Correlation of Degrees of Atherosclerosis Within and Between the Coronary and Cerebral Vascular Beds*

WEI YOUNG, PH.D., JOHN W. GOFMAN, M.D., F.A.C.C., ROBERT TANDY, Berkeley, California

NATHAN MALAMUD, M.D. and EUNICE S. G. WATERS, M.D.

San Francisco, California

Napa, California

YLINICAL and pathological observations have Joboth suggested the possibility that the coronary arterial system presents special problems with respect to involvement with atherosclerosis. Essentially this has centered around the type of case in which a serious clinical episode of coronary heart disease occurs during life and, later, autopsy reveals only isolated segments of the coronary arterial tree to be seriously involved with atherosclerosis. The remainder of the coronary vessels may be only minimally affected by atherosclerosis. Indeed such occurrences have at times led to the impression that atherosclerosis, or at least the type found in the coronary arteries, may not be part of a generalized disease. Yet, a vast body of evidence indicates that atherosclerosis, including coronary atherosclerosis, is a generalized process.1 It is, therefore, of importance to know in quantitative terms the true extent of relationship for atherosclerotic involvement among the coronary arteries. Further, it is of interest to know whether the interrelationships between the various coronary arterial branches represent a special case different from interrelationships within another important arterial bed, namely the cerebral arterial bed. Lastly, quantitative knowledge is desirable concerning the extent of correlation of degree of atherosclerosis between these two major arterial beds as part of an evaluation of the general character of atherosclerosis development. Young et al.2 have previously demonstrated that the degree of

atherosclerosis of the coronary arteries is significantly and positively correlated with the degree of atherosclerosis in the cerebral arteries. It is our purpose here to examine this relationship in more detail and to compare it with the extent of relationship of degree of atherosclerosis within each of these arterial beds.

MATERIAL AND METHODS

In the second paper of this seminar⁴ data were presented relating blood pressures during life in ninety-five subjects in whom the degree of coronary and cerebral atherosclerosis was later evaluated quantitatively at autopsy. This same group of ninety-five subjects serves as the study group for a measurement of the relationship of atherosclerosis between and within the coronary and cerebral arterial beds. Quantitative measurement of degree of atherosclerosis was made in the manner described previously³ and such degrees are reported below in units of I/E.

RESULTS

Atherosclerosis Within Arterial Beds: The extent to which the degree of atherosclerosis in any one major coronary arterial branch is related to that in other coronary branches is presented in the data of Table 1. In each case the mean degree of sclerosis for that arterial segment over all the cases in the series is presented, together with the correlation coefficient between degree in that branch and in other major branches.

The extent to which the degree of atherosclerosis in any one major cerebral arterial

^{*} From the Division of Medical Physics, Donner Laboratory of Medical Physics, Radiation Laboratory, University of California, Berkeley, California; the Langley Porter Clinic, San Francisco, California; and The Napa State Hospital, Napa, California. This work was supported by the Albert and Mary Lasker Foundation and the Samuel Roberts Noble Foundation and the U. S. Atomic Energy Commission.

Table I
Correlation of Degree of Atherosclerosis Between Major Branches of the Coronary Arteries

				I	Branches of C	oronary Artery		
Age Group (yr.)	Cases (no.)	Sclerosis and Inter- correlation	Left Main Coronary	Anterior Descending Branch	Left Anterior Branch	Left Circumflex Branch	Right Coronary	Average of all These Coronary Branches

A. Correlations Between Left Main Coronary Artery Versus the Other Branches

60-69	19	Mean sclerosis	39.1 ± 10.5	32.5 ± 14.5	28.3 ± 18.8	42.7 ± 14.0	39.3 ± 9.4	37.3 ± 9.5
		r*		0.13	0.47	0.15	0.49	0.46
		Significance test		N.S.	< 0.05	N.S.	< 0.05	< 0.05
70-79	49	Mean sclerosis	32.9 ± 13.3	37.6 ± 11.1	23.3 ± 10.4	38.3 ± 10.7	43.1 ± 10.0	36.9 ± 7.5
-		r*		0.18	-0.06	0.43	0.39	0.50
		Significance test		N.S.	N.S.	< 0.01	<0.05	~0.001
80-89	27	Mean sclerosis	34.2 ± 12.9	37.0 ± 16.8	29.2 ± 12.9	36.8 ± 9.4	38.0 ± 12.0	36.3 ± 8.6
		r*		0.29	-0.10	0.35	0.22	0.37
		Significance test		N.S.	N.S.	N.S.	N.S.	~0.05
60-89	95	Mean sclerosis	34.5 ± 12.9	36.4 ± 12.2	26.0 ± 13.4	38.8 ± 11.3	40.9 ± 10.8	36.8 ± 8.2
		r*		0.16	0.08	0.36	0.31	0.44
		Significance test		N.S.	N.S.	< 0.001	< 0.01	< 0.001

B. Correlation Between Anterior Descending Branch Versus the Other Branches

60-69	19	Mean sclerosis	 32.5 ± 14.5	28.3 ± 18.8	42.7 ± 14.0	39.3 ± 9.4	37.3 ± 9.5
		r*	 	0.6	0.58	0.41	0.85
		Significance test	 	< 0.01	< 0.01	N.S.	< 0.001
70-79	49	Mean sclerosis	 37.6 ± 11.1	23.3 ± 10.4	38.3 ± 10.7	43.1 ± 10.0	36.9 ± 7.5
		r*	 	0.40	0.46	0.36	0.73
- 1		Significance test	 	< 0.01	< 0.01	< 0.05	< 0.001
80-89	27	Mean sclerosis	 37.0 ± 16.8	29.2 ± 12.9	36.8 ± 9.4	38.0 ± 12.0	36.3 ± 8.6
		r*	 	0.29	0.85	0.71	0.90
		Significance test	 	N.S.	< 0.001	< 0.001	< 0.001
60-89	95	Mean sclerosis	 36.4 ± 12.2	26.0 ± 13.4	38.8 ± 11.3	40.9 ± 10.8	36.8 ± 8.2
		r*	 	0.40	0.54	0.47	0.80
		Significance test	 	< 0.001	< 0.001	0.001	< 0.001

C. Correlation Between Left Anterior Branch Versus the Other Branches

60-69	19	Mean sclerosis	 	28.3 ± 18.8	42.7 ± 14.0	39.3 ± 9.4	37.3 ± 9.5
		r*	 		0.41	0.23	0.76
		Significance test	 		N.S.	N.S.	< 0.001
70-79	49	Mean sclerosis	 	23.3 ± 10.4	38.3 ± 10.7	43.1 ± 10.0	26.9 ± 7.5
		r*	 		0.26	0.27	0.56
		Significance test	 		N.S.	N.S.	< 0.001
80-89	27	Mean sclerosis	 	29.2 ± 12.9	36.8 ± 9.4	38.0 ± 12.0	36.3 ± 8.6
-		r*	 		0.37	0.27	0.52
		Significance test	 		< 0.05	N.S.	< 0.01
60-89	95	Mean sclerosis	 	26.0 ± 13.4	38.8 ± 11.3	40.9 ± 10.8	36.8 ± 8.2
1		r*	 		0.33	0.20	0.60
		Significance test	 		~0.001	~0.05	< 0.001

TABLE I (Continued)

Correlation of Degree of Atherosclerosis Between Major Branches of the Coronary Arteries

			Branches of Coronary Artery							
Age Group (yr.)	Cases (no.)	Sclerosis and Inter- correlation	Left Main Coronary	Anterior Descending Branch	Left Anterior Branch	Left Circumflex Branch	Right Coronary	Average of all These Coronary Branches		
		D. Co.	rrelation Betwe	en Left Circumfle.	x Branch Vers	us the Other Bran	uches			
60-69	19	Mean sclerosis				42.7 ± 14.0	39.3 ± 9.4	37.3 ± 9.5		
		r*					0.38	0.80		
		Significance test		****	* * * *		N.S.	< 0.001		
70-79	49	Mean sclerosis	****			38.3 ± 10.7	43.1 ± 10.0	36.9 ± 7.		
		r*	* * * *	21.2.2.2	****		0.57	0.80		
		Significance test					< 0.001	< 0.001		
80-89	27	Mean sclerosis				36.8 ± 9.4	38.0 ± 12.0	36.3 ± 8.6		
		r*		* * * *			0.69	0.91		
		Significance test			****		< 0.001	< 0.001		
60-89	95	Mean sclerosis			* * * *	38.8 ± 11.3	40.9 ± 10.8	36.8 ± 8.2		
		r*	* * * *		* * * *		0.52	0.82		
	1	Significance test	****		• • • •		<0.001	<0.001		
		E. Con	relation Between	en Right Coronary	Artery Versus	s the Other Branc	hes			
60-69	19	Mean sclerosis					39 3 ± 9.4	37.3 ± 9.5		
			* * * *	****	* * * *		****	0.60		
70 70	40	Significance test					42 4 4 40 0	< 0.01		
70-79	49	Mean sclerosis					43.1 ± 10.0	36.9 ± 7.5		
			* * * *	****	* * * *	****	- ****	0.76		
	27	Significance test		* ** *			20 0 1 10 0	< 0.001		
30–89	27	Mean sclerosis					38.0 ± 12.0	36.3 ± 8.6		
		r*	****	****	* * * *		* * * *	0.86		
	0.5	Significance test					40 0 1 10 0	<0.001		
0 00		Baron colonomic					40.9 ± 10.8	36.8 ± 8.6		
50-89	95	Mean sclerosis						0 7/		
50-89	95	r* Significance test				****		0.74		

Note: 1* = Pearson coefficient of correlation.

branch is related to that in any other cerebral branch is presented in the data of Table II.

Atherosclerosis Between Vascular Beds: The extent to which the degree of atherosclerosis in the various cerebral arteries is related to that in the various major coronary arterial branches is presented in Table III.

COMMENTS

Several generalizations are possible concerning the extent of atherosclerosis in the coronary and cerebral arterial beds from examination of the data of Tables I, II and III. First, the overall degree of atherosclerosis is much greater, on the average, within the coronary arterial system than within the cerebral arterial system. This generalization is valid for each age group con-

sidered over the range from sixty to eighty-nine years. Indeed, the most severely involved of the cerebral regions, namely, the internal carotid and basilar arteries, shows a lesser average degree of sclerosis than the least severely involved coronary branch, namely, the left anterior coronary artery. Second, the degree of atherosclerotic involvement of any one coronary arterial branch is in general significantly and positively correlated with that of other coronary arterial branches.

Correlation of Involvement of Each Coronary Artery and Average of All Coronary Branches: The strength of the relationships can best be described by considering each individual coronary artery in comparison with the average for all coronary arteries studied. When this is

TABLE II
Correlation of Degree of Atherosclerosis Between Major Branches of the Cerebral Arteries

Age		Sclerosis		Bra	nches of Cerebra	al Artery	
Group (yr.)	Cases (no.)	and Inter- correlation	Anterior Cerebral Artery	Middle Cerebral Artery	Posterior Cerebral Artery	Carotid and Basilar Arteries	Average of all These Cerebral Arterial Branche
		A. Correla	ation Between Ant	erior Cerebral Art	tery Versus the Ot	her Branches	
60-69	27	Mean sclerosis	16.3 ± 12.4	19.9 ± 14.4	19.2 ± 14.6	22.9 ± 14.5	19.4 ± 13.4
		r*		0.90	0.89	0.87	0.95
70-79	49	Significance test Mean sclerosis	15.1 ± 11.3	$\ll 0.001$ 19.9 ± 13.4	$\ll 0.001$ 19.8 ± 12.9	$\ll 0.001$ 23.4 \pm 12.7	$\ll 0.001$ 19.5 ± 11.6
10-19	49	r*	15.1 2 11.5	0.85	0.78	0.91	0.88
		Significance test		≪0.001	<0.001	≪0.001	≪0.001
80-89	27	Mean sclerosis	13.5 ± 7.1	20.5 ± 13.2		21.7 ± 10.8	19.2 ± 10.4
		r*	* * * *	0.78 <0.001	0.83 ≪0.001	0.90 ≪0.001	0.96 ≪0.001
60-89	95	Significance test Mean sclerosis	14.9 ± 10.6	20.1 ± 13.6	20.2 ± 13.6	22.8 ± 12.6	19.4 ± 11.7
00 07	,,,	r*		0.83	0.78	0.92	0.89
		Significance test		≪0.001	<0.001	≪0.001	≪0.001
		B. Correl	ation Between Mi	ddle Cerebral Ari	tery Versus the Ot	ther Branches	
60-69	19	Mean sclerosis		19.9 ± 14.4	19.2 ± 14.6	22.9 + 14.5	19.4 ± 13.4
		r*			0.97	0.94	0.99
70-79	49	Significance test Mean sclerosis		19.9 ± 13.4	$\ll 0.001$ 19.8 ± 12.9	$\ll 0.001$ 23.4 ± 12.7	$\ll 0.001$ 19.5 ± 11.6
10-19	49	r*		19.9 ± 13.4	0.89	0.83	0.97
		Significance test			≪0.001	≪0.001	≪0.001
80-89	27	Mean sclerosis		20.5 ± 13.2	21.5 ± 13.8	21.7 ± 10.8	19.2 ± 10.4
		r*	****		0.84	0.74	0.93
60-89	95	Significance test Mean sclerosis		20.1 ± 13.6	$\ll 0.001$ 20.2 ± 13.6	<0.001 22.8 ± 12.6	$\ll 0.001$ 19.4 ± 11.7
00-89	. 93	r*		20.1 ± 15.0	0.89	0.83	0.96
		Significance test			≪0.001	≪0.001	≪0.001
		C. Correl	ation Between Pos	terior Cerebral Ar	rtery and the Other	r Branches	
60-69	19	Mean sclerosis			19.2 ± 14.6	22.9 ± 14.5	19.4 ± 13.4
		r*				0.89	0.98
70 70	40	Significance test			10 0 1 10 0	≪0.001	≪0.001
70-79	49	Mean sclerosis	* * * *	****	19.8 ± 12.9	23.4 ± 12.7 0.80	19.5 ± 11.6 0.95
		Significance test				< 0.001	≪0.001
80-89	27	Mean sclerosis			21.5 ± 13.8	21.7 ± 10.8	19.2 ± 10.4
		r*				0.78	0.96
60-89	95	Significance test			20 2 1 12 6	<0.001 22.8 ± 12.6	$\ll 0.001$ 19.4 ± 11.7
00-89	95	Mean sclerosis			20.2 ± 13.0	0.81	0.95
		Significance test				≪0.001	≪0.001
1	1	D. Correlatio	n Between Carotic	d and Basilar Art	eries Versus the O	ther Branches	
60-69	19	Mean sclerosis				22.9 ± 14.5	19.4 ± 13.4
		r*					0.97
70 =0		Significance test					≪0.001
713 703	49	Mean sclerosis				23.4 ± 12.7	19.5 ± 11.6 0.91
70-79		Significance test					≪0.001
10-19		Mean sclerosis				21.7 ± 10.8	19.2 ± 10.4
80-89	27	Mean scierosis					0 00
		r*		****		****	0.90
80-89	-	r* Significance test				22 0 + 12 6	≪0.001
		r*				22.8 ± 12.6	

Note: r* = Pearson coefficient of correlation.

Table
Correlations of Degree of Atherosclerosis Between

		0.1				
Age Group (yr.)	Cases (no.)	Sclerosis and Intercorrelation	Anterior Cerebral Arteries	Middle Cerebral Artery	Posterior Cerebral Artery	Basilar and Carotid Artery
4					A. Anter	ior Cerebral Arte
60-69	19	Mean sclerosis	16.3 ± 12.4			
		r*			****	
9	-	Significance test				
70-79	49	Mean sclerosis	15.1 ± 11.3			
		r*				
		Significance test				
80-89	27	Mean sclerosis	13.5 ± 7.1			
	-	r*				****
		Significance test			****	****
60-89	95	Mean sclerosis	14.9 ± 10.6			
		r*				
		Significance test			****	
'					B. Middle Cereb	ral Arteries Ver.
60-69	19	Mean sclerosis		19.9 ± 14.4		
		r*			****	
		Significance test				
70-79	49	Mean sclerosis		19.9 ± 13.4		
		r*				
		Significance test				
80-89	27	Mean sclerosis		20.5 ± 13.2		
		r*		****		
		Significance test	****		****	****
60-89	95	Mean sclerosis		20.1 ± 13.6		
		r*	****	****		
- 1		Significance test				

TABLE III Continued

done for the entire ninety-five subjects studied the following tabulation evolves:

	Correlation Coefficient (Pearson r)
Left circumflex branch versus average of	
all coronary branches	+0.82
Anterior descending branch versus av-	
erage of all coronary branches	+0.80
Right coronary artery versus average of	
all coronary branches	+0.74
Left anterior descending branch versus	
average of all coronary branches	+0.60
Left main coronary branch versus aver-	
age of all coronary branches	+0.44

It is clear from this tabulation that the degree of atherosclerosis in the left main coronary artery can deviate more from the over-all degree of

sclerosis of the coronary arteries than can any other major branch. Next in this ability to deviate from the over-all findings is the left anterior descending branch. Further, it can be stated that, within the coronary arterial bed, the relationships observed indicate the process of atherosclerosis to be in part generalized and in part, focal. Since the interbranch correlations are imperfect, it is to be expected that cases will be encountered relatively frequently in which one coronary branch is much more severely involved with atherosclerosis than any other branches. However, there is no conflict between the general and the focal character of the disease within the coronary arteries. The special features which characterize the left main coronary artery in comparison with all other coronary branches are of interest. The left main coronary artery is the shortest branch

Cerebral Arteries and Coronary Arteries

Major Bra	nches of Coronar	ry Arteries				
Average Cerebral Sclerosis	Left Main Coronary	Anterior Descending Branch	Left Anterior Branch	Left Circumflex Branch	Right Coronary	Average of all These Coronary Branche
rsus the Majo	or Coronary Branch	hes				
	39.1 ± 10.5	32.5 ± 14.5	28.3 ± 18.8	42.7 ± 14.0	39.3 ± 9.4	37.3 ± 9.5
	0.65	0.34	0.38	0.03	0.53	0.43
	< 0.001	N.S.	N.S.	N.S.	< 0.05	N.S.
	32.9 ± 13.3	37.6 ± 11.1	23.3 ± 10.4	38.3 ± 10.7	43.1 ± 10.0	36.9 ± 7.5
	0.22	0.31	0.24	0.54	0.44	0.52
	N.S.	< 0.05	N.S.	< 0.001	< 0.01	< 0.001
	34.2 ± 12.9	37.0 ± 16.8	29.2 ± 12.9	36.8 ± 9.4	38.0 ± 12.0	36.3 ± 8.6
	0.39	0.44	0.06	0.45	0.36	0.43
	< 0.05	< 0.05	N.S.	< 0.05	N.S.	< 0.05
	34.5 ± 12.9	36.4 ± 12.2	26.0 ± 13.4	38.8 ± 11.3	40.9 ± 10.8	36.8 ± 8.2
	0.33	0.32	0.23	0.38	0.42	0.47
	~0.001	<0.01	<0.05	<0.001	<0.001	< 0.001
Major Bran	ches of Coronary A	rteries		14		W 12 12 12 12 12 12 12 12 12 12 12 12 12
	39.1 ± 10.5	32.5 ± 14.5	28.3 ± 18.8	42.7 ± 14.0	39.3 ± 9.4	37.3 ± 9.5
	0.67	0.47	0.61	0.15	0.48	0.57
	< 0.001	< 0.05	< 0.01	N.S.	< 0.05	< 0.01
	32.9 ± 13.3	37.6 ± 11.1	23.3 ± 10.4	38.3 ± 10.7	43.1 ± 10.0	36.9 ± 7.5
	0.19	0.31	0.24	0.54	0.44	0.52
	N.S.	< 0.05	N.S.	< 0.001	< 0.01	< 0.001
	34.2 ± 12.9	37.0 ± 16.8	29.2 ± 12.9	36.8 ± 9.4	38.0 ± 12.0	36.3 ± 8.6
	0.41	0.33	0.20	0.38	0.37	0.43
	< 0.05	N.S.	N.S.	< 0.05	< 0.05	< 0.05
	34.5 ± 12.9	36.4 ± 12.2	26.0 ± 13.4	38.8 ± 11.3	40.9 ± 10.8	36.8 ± 8.2
	0.32	0.35	0.37	0.35	0.46	0.53
	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

on pages 306-307

of all those under consideration and is that branch (compared with all branches) which would probably be least affected by the motions of the heart. It is possible that the differing character of atherosclerotic development is a reflection of the effect of the motion of the heart upon the pressure operating with the various coronary arterial branches.

Correlation of Involvement of Each Cerebral Artery and Average of All Cerebral Branches: An entirely different picture emerges from the studies of the cerebral arterial branches. In the case of the cerebral arteries there exists no gross disturbing feature comparable with the motions of the heart, and it is observed that the interrelationship of degree of sclerosis between the various branches of the cerebral arteries is uniformly extremely strong. Indeed, within the experimental error of measurement, the

extent of correlation of degree of sclerosis in any one cerebral artery branch with degree of sclerosis in any other cerebral branch is nearly perfect. This is further reflected in the tabulation as follows:

	Correlation Coefficient (Pearson r)
Middle cerebral artery versus average of all cerebral branches	+0.96
Posterior cerebral artery versus average of all cerebral branches	+0.95
Carotid and basilar arteries versus average of all cerebral branches	+0,92
Anterior cerebral artery versus average of all cerebral branches.	+0.89

The strength of these correlations would

TABLE III
Correlations of Degree of Atherosclerosis Between

Age Group (yr.)	Cases (no.)	Sclerosis and Intercorrelation	Anterior Cerebral Arteries	Middle Cerebral Artery	Posterior Cerebral Artery	Basilar and Carotic Artery
					C. Posteri	or Cerebral Arter
60-69	19	Mean sclerosis			19.2 ± 14.6	
		r*				
		Significance test	* * * *	****		
70-79	49	Mean sclerosis			19.8 ± 12.9	
		r*			****	
		Significance test				
80-89	27	Mean sclerosis			21.5 ± 13.8	
		r*		****	****	****
		Significance test			00 0 1 40 6	
60-89	95	Mean sclerosis			20.2 ± 13.6	****
		r*	* * * *	****	* * * *	****
		Significance test		****		****
					D. Basilar a	and Carotid Arte
60-69	19	Mean sclerosis				22.9 ± 14.
		r*		****		
		Significance test				
70-79	49	Mean sclerosis				$23.4 \pm 12.$
		r*			*	
		Significance test				
80-89	27	Mean sclerosis				$21.7 \pm 10.$
		r*	****	****		****
	0.7	Significance test				22 0 1 12
60-89	95	Mean sclerosis				$22.8 \pm 12.$
		r*				
		Significance test	****	****	****	* * * *
					E. Averag	e Cerebral Sclero
60-69	19	Mean sclerosis	****			
		r*	* * * *			
		Significance test			****	
70-79	49	Mean sclerosis		****	****	
		r*			*	****
		Significance test			****	****
80-89	27	Mean sclerosis				
		r*	****			
40.00		Significance test		****	****	* * * *
60-89	95	Mean sclerosis			****	
		r*				
		Significance test			****	

Note: r* = Pearson coefficient of correlation.

indicate that a serious degree of involvement of any cerebral branch with atherosclerosis almost always bespeaks a correspondingly serious degree of involvement of all other cerebral branches. Therefore, the occurrence of isolated marked focal disease within the brain supply should be rare compared with the occurrence of isolated focal atherosclerotic involvement of a coronary artery. Since this is true, the over-all clinical implication of manifest athero-

(Continued)
Cerebral Arteries and Coronary Arteries

Major Bra	nches of Corona	ry Arteries				
Average Cerebral Sclerosis	Left Main Coronary	Anterior Descending Branch	Left Anterior Branch	Left Circumflex Branch	Right Coronary	Average of all These Coronary Branche
rsus the Majo	or Coronary Branch	hes				Media.
	39.1 ± 10.5	32.5 ± 14.5	28.3 ± 18.8	42.7 ± 14.0	39.3 ± 9.4	37.3 ± 9.5
	0.63	0.40	0.46	0.20	0.53	0.52
	~0.001	N.S.	< 0.05	N.S.	< 0.05	< 0.05
	32.9 ± 13.3	37.6 ± 11.1	23.3 ± 10.1	38.3 ± 10.7	43.1 ± 10.0	36.9 ± 7.5
	0.27	0.27	0.25	0.59	0.60	0.60
	N.S.	N.S.	N.S.	< 0.001	< 0.001	< 0.001
	34.2 ± 12.9	37.0 ± 11.8	29.2 ± 12.9	36.8 ± 9.4	38.0 ± 12.0	36.3 ± 8.6
	0.34	0.35	0.10	0.31	0.17	0.30
	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	34.5 ± 12.9	36.4 ± 12.2	26.0 ± 13.4	38.8 ± 11.3	40.9 ± 10.8	36.8 ± 8.2
	0.34	0.33	0.27	0.39	0.42	0.48
	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001
	<0.001 32.9 ± 13.3 0.31 <0.05 34.2 ± 12.9 0.49 <0.01 34.5 ± 12.9 0.42 <0.001	<0.05 37.6 ± 11.1 0.22 $N.S.$ 37.0 ± 11.8 0.53 <0.01 36.4 ± 12.2 0.35 <0.001	<0.01 23.3 ± 10.4 0.27 N.S. 29.2 ± 12.9 0.07 N.S. 26.0 ± 13.4 0.30 <0.01	N.S. 38.3 ± 10.7 0.60 <0.001 36.8 ± 9.4 0.59 <0.001 38.8 ± 11.3 0.46 <0.001	<0.01 43.1 ± 10.0 0.65 <0.001 38.0 ± 12.0 0.44 <0.05 40.9 ± 10.8 0.55 <0.001	<0.01 36.9 ± 7.5 0.60 <0.001 36.3 ± 8.6 0.53 <0.01 36.8 ± 8.2 0.57 <0.001
sus the Majo	r Coronary Branch	es				
.4 ± 13.4	39.1 ± 10.5	32.5 ± 14.5	28.3 ± 18.8	42.7 ± 14.0	39.3 ± 9.4	37.3 ± 9.5
	0.69	0.42	0.51	0.12	0.54	0.53
	<0.001	N.S.	< 0.05	N.S.	<0.05	< 0.05
$.5 \pm 11.6$	32.9 ± 13.3	37.6 ± 11.1	23.3 ± 10.4	38.3 ± 10.7	43.1 ± 10.0	36.9 ± 7.5
* * * *	0.25	0.29	0.31	0.58	0.62	0.61
	N.S.	< 0.05	< 0.05	< 0.001	< 0.001	< 0.001
$.2 \pm 10.4$	34.2 ± 12.9	37.0 ± 16.8	29.2 ± 12.9	36.8 ± 9.4	38.0 ± 12.0	36.3 ± 8.6
	0.43	0.44	0.12	0.44	0.34	0.44
	< 0.05	< 0.05	N.S.	< 0.05	N.S.	< 0.05
$.4 \pm 11.7$	34.5 ± 12.9	36.4 ± 12.2	26.0 ± 13.4	38.8 ± 11.3	40.9 ± 10.8	36.8 ± 8.2
	0.37	0.36	0.31	0.41	0.50	0.54

sclerotic disease in any one region of the cerebral bed is more serious than manifest clinical disease in a single coronary branch.

Correlation of Cerebral and Coronary Artery Involvement: Lastly, the data in Table III

confirm the general character of the development of atherosclerosis in showing that correlation of the degree of sclerosis of the various branches of the cerebral arteries with the degree in the coronary arteries is significant and positive. The strength of this interarterial bed relationship is much less than the strength of the relationship between degrees of sclerosis within the cerebral bed, but of the same order of strength as that within the coronary arterial bed. Thus, although the evidence clearly supports the general aspect of the development of atherosclerosis, the imperfection of the "between-bed" correlations allows for the expectation, in individual cases, of serious sclerosis within the coronary arterial bed without correspondingly severe involvement of the cerebral arteries.

SUMMARY

1. The strength of the relationship of the degree of atherosclerosis within major branches of cerebral arteries (anterior cerebral branch, middle cerebral branch and carotid and basilar branches) is much stronger than those within coronary arteries (left main coronary, anterior descending branch, left anterior branch, left circumflex and right coronary).

2. The strength of interrelation of athero-

sclerosis between coronary and cerebral arterial branches is much less than that within the cerebral arterial beds but of a lesser order of magnitude than that within the coronary arterial beds. Thus the extent of correlation of the degree of atherosclerosis within and between the coronary and cerebral vascular beds may be represented as follows: Within cerebral beds> within coronary beds> between coronary and cerebral beds.

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Digitalis, Electrolytes and the Surgical Patient*

Bernard Lown, M.D., Harrison Black, M.D. and Francis D. Moore, M.D.

Boston, Massachusetts

The INCREASE in cardiac as well as in extracardiac operations performed on patients with heart disease poses a number of problems regarding the proper use of the digitalis drugs. The objective of digitalis therapy is the same whether or not a surgical procedure is to be undertaken: to achieve and maintain optimal cardiac compensation while avoiding the hazards of digitalis intoxication. Anesthesia and surgical procedures, however, dislocate a number of bodily processes that affect the myocardial response to digitalis. Over- or underdigitalization may result. Recovery depends upon prompt recognition and correction of either of these conditions.

There are three phases of the operative experience in which the relative state of digitalization may be altered. First, the phase of anesthesia, in which the major hazard is anoxia with or without hypercarbia. Other factors of importance are the anesthetic agent itself, the preanesthetic medications, the vagal reflexes produced by tracheal intubation and suction and the Valsalva maneuver, which may accompany induction of anesthesia, tracheal intubation or coughing. Second, the operation itself, even though not directed to the heart, may alter the circulation through changes arising from manipulation of tissues, from hypotension and shock, from tissue anoxia provoked by loss of blood or hypoventilation or from the excessive or inadequate replacement

of fluids. Finally, in the immediate postoperative period the metabolic consequences of trauma are characterized by internal shifts in the concentration of sodium and potassium and by dilutional hypotonicity. These changes are prone to be especially severe in the patient with heart failure and may alter the cardiac threshold to digitalis.

During the past decade it has become clear that the action of digitalis is influenced by alterations in body electrolytes. More recent findings suggest that digitalis, in turn, influences the concentration of electrolytes in certain body fluids. Since the patient who undergoes surgery experiences shifts in electrolyte content and concentration, it is pertinent to examine the indirect effects of the surgical procedure on the action of digitalis. This report, therefore, will deal with the effect of alterations of individual ions on the action of digitalis; the influence of digitalis on the serum concentration of sodium, potassium and calcium; the metabolic and electrolyte alterations resulting from operation; and finally will explore some outstanding problems in the use of digitalis drugs in patients in heart failure undergoing surgical procedures.

GENERAL CONSIDERATION OF DIGITALIS ACTION

There are no reported animal or human studies dealing with the multiple and complex effects of operative trauma on the digitalized

^{*} From the Department of Nutrition, Harvard School of Public Health, and the Departments of Surgery and Medicine, Peter Bent Brigham Hospital, Boston, Massachusetts. These studies were supported in part by grants-in-aid to the Department of Nutrition from the Fund for Research and Teaching, Department of Nutrition, the National Heart Institute (H-2200), National Institutes of Health, Bethesda, Maryland, and to the Department of Surgery from the Atomic Energy Commission and the United States Army through the Office of the Surgeon-General.

state. In fact, the principles that guide the present-day clinical use of digitalis are empirical and are based in part on the conclusions for-

mulated by Withering 175 years ago.

The cardiac glycosides have the common capacity to alter excitability, conductivity and contractility of heart muscle (the positive ionotropic action). These actions have been employed in three clinical settings: (1) to control and prevent paroxysmal ectopic arrhythmias of atrial or nodal origin; (2) to reduce atrioventricular conduction in atrial flutter or fibrillation; and (3) to increase ventricular contractility of the failing heart. The last-named effect is the most important pharmacologic property of the

digitalis drugs.

The full beneficial effects of the administration of digitalis are derived only after the dosage requirements of the individual patients are fulfilled. To date there exists no effective method for predicting these needs in any one patient. Requirements may vary widely among patients and in any one patient at different times. The use of digitalis demands meticulous attention to the patient's response. Few drugs in current use have as narrow a margin of safety. Once full digitalization has been achieved, 60 per cent of the potentially toxic dose already has been administered. Once toxicity develops, the patient has received about 40 per cent of the minimum lethal dose. This narrow therapeutic-toxic ratio is further reduced by old age, by advanced congestive heart failure, by the concomitant use of other drugs and in the presence of certain electrolyte derangements. Digitalization therefore requires the administration of repeated small doses. In effect it constitutes a "biological titration" to determine the patient's requirement for a maximum therapeutic effect.

As the patient with heart disease approaches a surgical operation, whether or not it is an operation on the heart itself, there are four basic questions that must be answered relative to digitalization. (1) Should the patient be digitalized and if so, when? (2) How rapidly should digitalization be accomplished and with what drug? (3) If already digitalized, has maximum digitalis effectiveness in terms of myocardial efficiency been achieved, and if ideal digitalization does not exist, is there over- or underdigitalization? (4) During the intraoperative and postoperative course, what events may transpire that alter the effectiveness of whatever digitalis is already present in the body?

Preoperative digitalization should be carried out in patients who are in congestive heart failure, in patients currently compensated but who have previously been in heart failure and in patients with atrial fibrillation or flutter. The patient over sixty years of age who has an enlarged heart, even in the absence of overt signs of congestive failure, deserves prophylactic digitalization. Once the decision has been made regarding the need for digitalis, administration should not be postponed until the night prior to operation or deferred until such action is forced by the development of congestive heart failure in the intraoperative and immediate postoperative period. Ideally, the surgical patient should be digitalized orally in the week or two prior to operation. This provides time to assess individual tolerance for the drug.

The rapidity of digitalization depends on whether it is an elective procedure or one dictated by the unexpected emergence of heart failure or arrhythmia. In the surgical patient acute changes in body fluid environment, in cardiovascular volume, and pressure and rate loads, as well as in myocardial excitability, may predispose to special vulnerability to digitalis. A rapidly dissipated drug such as digoxin is, therefore, generally preferred. Both oral as well as the more rapid parenteral digitalization can be effected with this agent. Whenever possible, intravenous digitalization is to be avoided since the heart becomes exposed to a sudden high concentration of the agent which may precipitate serious arrhythmia or death. In the absence of an emergency situation, but when the drug cannot be administered orally, it should be administered intramuscularly.

The determination of the end point of digitalization frequently presents a problem. In the presence of atrial fibrillation the ventricular rate serves as an accurate yardstick of adequate dosage. The absence of pulse deficit, and a ventricular rate below 80 per minute which does not accelerate to over 100 per minute on mild exercise, are useful indices. When the patient is in normal sinus rhythm the heart rate is not a reliable guide to the adequacy of digitalization. Disappearance of such symptoms as dyspnea and orthopnea and the clearing of objective stigmas of cardiac decompensation are the goals of therapy. At times it is necessary to digitalize to mild toxicity before the patient's specific requirement can be accurately determined. Electrocardiographic changes, such as inversion of the initial portion of the T wave, depression

of the S-T segment and shortening of the Q-T interval, commonly referred to as "digitalis effect," bear no relation to the optimal therapeutic dose.

Anesthesia and operation may uncover underdigitalization or increased cardiac sensitivity to the drug. The experience with many patients undergoing mitral valve surgery has demonstrated a frequent need for the administration of additional digitalis in the immediate postoperative period. The increased requirement may be the result of numerous factors such as fever, a rise in metabolic rate, the onset of atrial fibrillation or dilutional hypotonicity.

Surgical operation may also precipitate overdigitalization. The problem is compounded by the fact that in the cardiac patient the manifestations of underdigitalization may simulate those of overdosage. Nausea and vomiting result from the increased visceral congestion of heart failure as well as from digitalis intoxication. Similarly, ventricular ectopic beats, bigeminy and even ventricular tachycardia may be occasioned by either of these. It has long been assumed that digitalis does not accelerate atrial pacemakers. Recent observations,2 however, indicate that atrial tachycardia is a common manifestation of digitalis intoxication. The prototype of the digitalis-induced atrial arrhythmias is paroxysmal atrial tachycardia with atrioventricular block. These disorders generally represent far advanced intoxication. The development of a rapid arrhythmia during or after operation may be due to a sinus or atrial tachycardia or indeed be paroxysmal atrial tachycardia with atrioventricular block. It is currently taught that digitalis is the drug of choice in the treatment of atrial tachycardias. Obviously, its use to combat digitalis-induced atrial arrhythmias may further compromise myocardial function and even jeopardize survival. The digitalis-provoked atrial disorders frequently develop on the basis of shifts in electrolytes without a change in the maintenance schedule of digitalis. Although, as already noted, requirements of digitalis are commonly increased after surgery, this form of overdosage arrhythmia may make its appearance when a large postoperative diuresis occurs or digitalis dosage is unwisely increased in an attempt to slow a sinus tachycardia.

How then do changes in body electrolytes affect the action of digitalis? To date only the effects of alterations of individual cations have been studied. It must be emphasized that

"isolated" electrolyte changes are more a figure of speech than a matter of fact. The entire electrolyte structure is necessarily strained when the concentration of one of the components is changed. Cardiac effects are, therefore, not the consequence of the isolated addition or removal of a single ion but rather the result of the modification in the total organism following the single alteration.² It is in this sense that the observations to be described need be considered.

POTASSIUM AND DIGITALIS

INCREASE IN BODY POTASSIUM

The varying myocardial sensitivity to digitalis is determined in part by the balance of potassium in the body. Administration of potassium protects the heart against digitalis-induced arrhythmias.3 In dogs the level of serum potassium can be raised and maintained relatively constant by means of hemodialysis.* When the serum potassium level is fixed between 7 and 8 mEq. per L. the dose of digitalis required for a toxic effect is increased approximately 240 per cent. Ventricular ectopic beats are of brief duration if they occur. At these high serum potassium concentrations, digitalization generally will produce an electrocardiographic pattern of progressive hyperkalemia with death resulting from cardiac standstill, rather than provoke the usual stigmas of digitalis overdos-

In animals and man administration of potassium generally controls all disorders of rhythm and conduction precipitated by administration of digitalis. This effect of potassium apparently does not interfere with the therapeutic action of digitalis. Studies on the isolated perfused heart indicate that, while potassium antagonizes the toxic effects, it will not block the increase in contractility resulting from the addition of ouabain. Conversely, digitalis glycosides may inhibit, delay or overcome heart failure precipitated by potassium intoxication in the heart-lung preparation of the dog.⁷

It is important to know whether or not administered potassium alters the lethal as well as the toxic threshold to digitalis. If

^{*} In this and the succeeding four sections of the paper experimental data from the dog are mentioned. Such data are unobtainable in man because of the prohibitive risk of such drastic changes in cardiac function. Species differences in digitalis action do not appear to be prominent although their possibility is acknowledged.

this were not the case, the use of potassium would merely abolish the warning signs of an impending lethal overdose of digitalis. Experiments in dogs⁸ indicate that following potassium administration, 40 per cent more digitalis is required for lethal action than in animals which did not receive potassium.

It has long been recognized that small changes in concentration of potassium have profound effects on excitability and electrical activity of both isolated hearts and hearts in situ, especially when the level of calcium remains constant.9 Regardless of cause, ventricular ectopic beats can usually be eliminated by the administration of potassium. 10,11 It is possible that the abolition of digitalis arrhythmia by administration of potassium is in the nature of a non-specific pharmacologic effect. A number of observations support the conclusions that (1) administration of potassium controls ventricular arrhythmias whether induced by digitalis or not; (2) the amount of potassium required for a therapeutic effect is the same irrespective of the etiology of the arrhythmia; and (3) the suppression of the ectopic rhythms may be transient and persist only during the brief phase of hyperkalemia. Studies of the action of digitalis in the presence of potassium deficits, however, suggest that the relation is physiologic as well as pharmacologic.12

DECREASE IN BODY POTASSIUM

Depletion of body potassium stores sensitizes the myocardium to the toxic action of digitalis. In dogs the selective removal of potassium by means of hemodialysis has been associated with striking changes in the myocardial threshold to digitalis.3 When 5 to 10 per centrof a dog's total body potassium was removed and the serum concentration lowered to 2.0 mEq. per L., only 40 per cent of the control toxic dose of digitalis was required to produce ventricular tachycardia. The resulting toxic rhythms consisted of bigeminal and multiform ventricular disorders, mechanisms unusual in the absence of electrolyte derangement. These continued for a longer time than the usual duration of drug action. Frequently, resolution of the arrhythmia followed only on restitution of potassium stores. Furthermore, the potassium-depleted animal was sensitive to provocation of digitalis intoxication by anoxia and circulatory loading. Profound and prolonged arrhythmias followed the rapid injection of as little as 50 ml. of blood.

Loss of Potassium Following Diuresis: An

loss of potassium in patients sensitizes the heart to digitalis intoxication. It has long been known¹⁸ that following mercurial-induced diuresis, all the stigmas of digitalis overdosage may develop in the digitalized cardiac patient. This reaction to diuretics has been attributed to cardiac "redigitalization" through the mobilization of digitalis-laden edema fluid.14 Extracellular fluid, however, contains insignificant concentrations of digitalis, quantities hardly sufficient to produce overdosage even after profuse diuresis.15 A more likely explanation is that the postdiuretic syndrome represents a state of increased myocardial sensitivity to the toxic properties of digitalis resulting from a negative potassium balance precipitated by diuretic therapy.12 This supposition is confirmed by the observation, on the one hand, that manifestations of overdosage occur only when significant losses of potassium accompany the diuresis and, on the other, that when these losses are replaced, the mercurial diuretics do not potentiate the toxic action of digitalis.16

The tendency to loss of potassium after various diuretics may be enhanced by prolonged and rigid restriction of salt intake.17 In patients with a good capacity for sodium excretion, as is true in the early phases of heart failure, even extensive diuresis does not result in significant loss of potassium. Potassium is believed to participate in the distal tubular exchange process.¹⁸ When sodium is in short supply this mechanism may be activated for the conservation of cations. The renal tubular cells secrete potassium and hydrogen ions in exchange for sodium ions in the tubular urine. After long continued diuretic therapy, such preferential losses of potassium may lead to cumulative and clinically significant deficits of this cation, occasionally resulting in hypochloremic and hypokalemic alkalosis. Potassium losses are further enhanced by the administration of ammonium chloride prior to the mercurial agents to enhance diuretic response. Administration of acetazoleamide, the cationic exchange resins, chlorothiazide and hydrochlorothiazide may also induce serious potassium deficits. In the majority of patients in advanced congestive heart failure the loss of potassium is from the great mass of intracellular potassium and cannot be detected by determining the serum level, which is often elevated despite bodily depletion. 19-21

The myocardial threshold to digitalis may also be reduced by a shift of potassium from the

TABLE I

Relation Between Digitalis Dose Necessary to Produce Ventricular Tachycardia, Body Weight and Serum Electrolyte
Concentrations in Two Groups of Dogs

Group	No. of	Mean Toxic Dose of Acetyl Strophanthidin	Mean Weight	Serum Electrolytes (mEq./L.)		
Group	Digitalizations	(mg.)	(kg.)	K+	Na ⁺	Ca++
1.	20	1.21 ± 0.15	14.8 ± 1.4	4.58 ± 0.25	148.9 ± 3.2	5.53 ± 0.15
II	20	0.604 ± 0.12	14.7 ± 1.9	4.53 ± 0.22	148.3 ± 3.0	5.35 ± 0.19

Note: Despite the similarity in weights and serum electrolyte concentrations, one group required twice the amount of acetyl strophanthidin to produce toxicity.

extracellular to the intracellular compartment without any loss of this cation from the body. Thus, digitalis intoxication may follow the rapid lowering of the serum potassium by the administration of sodium chloride, sodium bicarbonate, glucose or insulin. It has been shown^{2,22} that in the critically digitalized patient, oral administration of carbohydrates or intravenous administration of glucose or insulin may precipitate ventricular premature beats or ventricular tachycardia.

Serum Potassium and the Myocardial Threshold to Digitalis: Does the myocardial threshold to digitalis, therefore, depend on the serum potassium concentration? Available evidence does not support this thesis. (1) In animals, the quantity of digitalis necessary to produce cardiac arrhythmias bears no relation to the serum potassium concentration. Two groups of dogs, similar as to weight, sex and age, but one requiring twice the dose of acetyl strophanthidin to produce ventricular tachycardia were compared. No difference in their serum electrolyte concentrations was observed (Table 1). (2) In experimental studies, maintenance of a constant but reduced serum potassium concentration during extraction of potassium by hemodialysis increased myocardial sensitivity to digitalis. In effect progressively less digitalis was necessary to produce intoxication although the serum potassium level remained relatively constant at a low level.23 (3) Digitalis intoxication is not associated with a reduction in serum potassium concentration. This is true even when the cardiac arrhythmia has resulted from renal potassium loss following diuresis. (4) The abolition of digitalis intoxication by potassium administration is not related to any sustained rise in the serum potassium concentration. (5) When a cardiac arrhythmia develops during the removal of

potassium by hemodialysis, restoration of the initial serum concentration will not promptly control the disordered mechanism. At the time of reversion to normal rhythm the serum potassium concentration may indeed have receded to a level lower than that which existed during the arrhythmia.

Myocardial Potassium Gradient: The myocardial threshold to digitalis intoxication is in part determined by the body content of potassium relative to other cell solids. This threshold decreases with a negative potassium balance and, within limits, increases with a positive balance. The serum potassium concentration is only indirectly related to this threshold. The underlying factor may be the potassium concentration within the myocardial cell or the potassium gradient. Deficit within the myocardium may thus enhance cardiac sensitivity even without external losses. For example, when glucose and insulin are administered, the potassium deposited with glycogen is thereby sequestered and removed from participation in muscular contraction. This may impose a deficit on tissues such as the heart. In this instance, also, the myocardial potassium concentration, or more specifically, the gradient of potassium across the cell membrane may be the critical factor.

Serum Sodium: There is no knowledge of the effects of changes in serum sodium concentration, serum osmolality, or blood pH on the cardiac action of digitalis in patients with heart disease. In experimental studies, changes in sodium concentration do not appear to influence the excitability properties of heart muscle. Alteration in the concentration of extracellular sodium has little effect on the resting potential of Purkinje fibers. Decrease in sodium concentration does not alter the resting potential of the ventricle of the frog. 24b Only

when the sodium concentration is reduced to 25 per cent of normal is there alteration in the excitability properties of the heart of the guinea pig.²⁵ Change in sodium concentration, however, does alter cardiac contractility.²⁶

CALCIUM AND DIGITALIS

While the effects of isolated changes in body potassium content on the reaction of the heart to digitalis administration have been considered, it is evident that in the intact organism the myocardial response to digitalis is determined by the continuous interaction of numerous ionic and non-ionic constituents. There is a complex interplay of forces in the medium, and rarely does a single element exhibit an isolated charge. The role of calcium in influencing digitalis action has long been recognized. A relation between calcium and digitalis as well as a similarity in their actions has been found on both the contractility and excitability properties of heart muscle.

Effect on Cardiac Contractility: Ringer's classic studies27,28 demonstrated that if contraction of heart muscle is to be sustained for more than a few minutes three cations, sodium, potassium and calcium, are essential constituents of a perfusion medium. Chloride is the only essential anion. For prolonged activity the incorporation of bicarbonate and phosphate ions is beneficial. When the level of calcium in the perfusate of the heart of the frog is increased, a more vigorous systole ensues. This effect of calcium is opposed by raising the concentrations of potassium. A simple antagonism between the two ions on the development of myocardial tension probably is inadequate to explain the data so far recorded. For example, the heart of the frog, suspended in a nutrient medium, contracts maximally at a specific potassium concentration of 4.8 mM per L.29 Variation from this value, irrespective of direction, causes a sharp reduction in contractility. The magnitude of contraction at any given potassium level, however, is determined simultaneously by the concentration of calcium. As the level of calcium is increased, contractility is enhanced, with the peak of contractility occurring only at the optimal potassium level. In the isolated heart of the frog, calcium and digitalis act synergistically to increase contractility. The digitalis-induced change is similar to that resulting from the addition of calcium. In effect, the glycosides behave as though substituted for a certain quantity of calcium.29

Effect on Cardiac Excitability: The similarities between the actions of calcium and digitalis are also observed on the excitability of the heart. It has been emphasized earlier that increase of potassium will prevent digitalis-induced arrhythmias while a decrease of potassium will sensitize the heart to such disorders. This relation holds if calcium is substituted for digitalis. In the isolated heart of the rabbit ventricular fibrillation can be initiated by rapid injection of calcium or by perfusion with a potassium-free solution.30 These are related phenomena. Calcium injection does not result in fibrillation when the potassium concentration exceeds 2.3 mEq. per L., or is a potassium-free solution effective when calcium concentration is less than 3.5 mEq. per L. A relation between calcium and digitalis has also been observed on the transmembrane potentials of the individual myocardial fiber. 31 Microelectrodes placed in a single myocardial fiber of an isolated, perfused electrically driven ventricle of the frog revealed a shortening of the transmembrane action potential within three hours following digitalization. Doubling the extracellular calcium concentration decreased digitalization time to one hour. When calcium was excluded from the perfusate, the typical digitalis effects did not develop. In a fully digitalized heart, on withdrawal of calcium, the action potential reverted to normal within one minute.

The effect of calcium on the configuration of the transmembrane action potential of the isolated atrial muscle fiber resembles that of acetylcholine.9 When calcium is administered to animals or men, the initial effects are bradycardia, reduction in P wave amplitude, sinus arrhythmia, shifting pacemaker and P-R interval prolongation. 32,33 These actions are abolished by administration of atropine. Similarly, the initial effects of digitalis are vagal and consist of a reduction in size of the P wave and slowing of the heart rate. These changes may also be abolished by administration of atropine. The rapid intravenous infusion of large doses of calcium results in ventricular ectopic beats of large and bizarre shape. Like digitalis, calcium abbreviates the Q-T interval.

Mechanism of Calcium and Digitalis Effects on the Myocardium: It is inviting to speculate that the similarity and interrelation in the biologic effects of digitalis and calcium stem from a similar action upon the cell membrane. Changes in the electrical properties of the myocardial cell

are ascribed to alterations in membrane permeability to potassium and sodium ions. The calcium ion participates in determining the electrical stability of the polarized cell membrane. The magnitude, rate and direction of exchange of cations during depolarization and repolarization of excitable tissue, depends on the concentration of ionized calcium. Increasing the calcium concentration augments the capacity of the myocardial fiber membrane to change its sodium permeability during depolarization. Conversely, lowering the calcium concentration decreases permeability of sodium.34 The transmembrane potentials obtained from the individual fiber of the frog's ventricle during perfusion with low concentrations of sodium closely resemble those of the digitalized heart. These revert to normal immediately after the removal of calcium.³¹ Forty-five years ago, Clark²⁶ noted that by decreasing the concentration of extracellular sodium, an increase in the contractility of heart muscle resulted. According to Szent-Györgyi³⁵ and Hajdu,³⁶ the contractile force of the myocardium depends on the total quantity of intracellular sodium and potassium. It may be that the changes in excitability and contractility promoted by both digitalis and calcium are but expressions of an altered intracellular electrolyte environment.

The aforementioned considerations indicate a relation between digitalis and calcium. The clinical meaning and quantitative implications cannot be derived from studies of the isolated heart. It is evident that a response of the total organism to changes in calcium or to digitalization may induce alterations which in turn amplify, modify or annul the interaction between the two observed in the isolated heart.

The effective moiety of calcium is the ionized fraction. At the present time this fraction cannot readily be measured. With this qualification it is worth examining the effect of raising and lowering the calcium concentration upon the digitalized state in the intact organism.

INCREASE IN SERUM CALCIUM

Experimental Effects: The results of administering calcium to digitalized animals have given rise to widely contradictory interpretations. These are due to the failure to appreciate a number of facts: (1) Large doses of calcium alone may evoke arrhythmias similar in some respects to those resulting from digitalis intoxication. (2) Calcium is rapidly and variably cleared from the blood.³⁷ (3) The calcium ef-

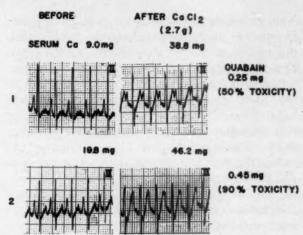


Fig. 1. Rapid intravenous administration of calcium does not provoke arrhythmias in the partially or fully digitalized animal. Strip 1, in a dog digitalized to 50 per cent of toxicity, infusion of 2.7 gm. calcium over thirty minutes raises the serum concentration to 38.8 mg. per 100 ml. yet does not produce ventricular arrhythmia. Strip 2, when the animal is digitalized two hours later to 90 per cent of toxicity, administration of a similar amount of calcium again fails to provoke digitalis intoxication, although the serum calcium concentration reaches 46.2 mg. per 100 ml.

fect on the heart is due to the ionized fraction. (4) The level of the ionized fraction is determined by the pH, the quantity and pattern of serum proteins, and the level of parathormone. (5) Cardiac effects of calcium are conditioned by other ions, specifically magnesium and potassium. (6) Calcium administration, when carried out by syringe injection or intravenous drip infusion, as has been the case in nearly all reported studies, gives rise to unpredictable results; furthermore, in previous studies the state of digitalization prior to the administration of calcium was not accurately determined or controlled.

In recent experiments in dogs a conscientious attempt was made to overcome these objections.38 Animals served as their own controls. Each study was completed in a single day. Electrolyte, pH, serum protein and hematocrit values were observed. Ventilation was maintained constant. Calcium was administered by a constant infusion pump. The state of digitalization was repeatedly determined. In these studies no demonstrable sensitivity to calcium was found when the degree of digitalization varied from zero to 90 per cent of toxicity. Digitalis-like arrhythmias were provoked by calcium only when the animals had received in excess of 95 per cent of the toxic dose of ouabain. Some of the results of a representative experiment are illustrated in Figure 1.

The experimental procedure was as follows: Dogs were digitalized with acetyl strophanthidin to an end point of ventricular tachycardia. On recovery, generally occurring within ten minutes, ouabain in a dose equivalent to half the toxic dose of acetyl strophanthidin was administered. 12,39 To check the accuracy of this estimate, retitration with acetyl strophanthidin was carried out. The difference between the first and second doses of acetyl strophanthidin represented the effective ouabain in the body. Calcium chloride was then injected intravenously by means of a constant infusion pump until either arrhythmia was produced or a total dose of 2.7 gm. was given in thirty minutes. One hour after the aforementioned an additional increment of ouabain was given to raise the dose to 90 per cent of the toxic level. This was again checked with acetyl strophanthidin. Calcium was then readministered. In the experiment illustrated (Fig. 1), raising the serum calcium to 46.2 mg. per 100 ml. in the presence of an advanced degree of digitalization did not precipitate any digitalis arrhythmia. These results are in accord with some earlier reports. 40,41 When the calcium concentration was maintained at 25 mg. per 100 ml. by means of parathormone injection, the sensitivity to digitalis was not increased.40 The lethal dose of digitalis in dogs was reduced only when large doses of calcium were given to the point of electrocardiographic derangement.

Clinical Effects: The generally accepted clinical view of a synergism between calcium and digitalis on the excitability of the human heart is based in part on a single but widely quoted report⁴² of two digitalized patients who died shortly after receiving small amounts of calcium intravenously. However, no deaths have been reported from the extensive use of calcium gluconate to determine the circulation time. This test has been frequently employed in critically digitalized patients. More recent studies purporting to show a quantitative additive relation between calcium and digitalis are based on uncontrolled experiments inadequate

in design to answer this question. 43-45

It may be concluded that in the intact animal a calcium-digitalis synergism on cardiac excitability cannot be demonstrated. When digitalization is carried to the brink of toxicity or when calcium is administered to the point of electrocardiographic derangement, an additive interaction is evident. As yet unanswered is the question of whether or not a calcium-digitalis

synergism exists in the presence of heart failure. Another unresolved question is whether or not calcium and digitalis act additively in the intact organism to increase cardiac contractility.

DECREASE IN SERUM CALCIUM

Reduction in the fraction of ionized calcium is not an uncommon problem in the surgical patient. This is most frequently due to the administration of citrated blood. Hypocalcemia has also been observed with acute necrotizing pancreatitis46 and reduction in diffusible calcium following abdominal and thoracic surgery, frequently in association with large citrated blood transfusions.47 Lowering the serum calcium concentration may compromise cardiac function. For example, when animals are bled and then retransfused with either heparinized or citrated blood, in those receiving the citrated replacement hypotension develops which can be corrected only by administration of calcium.48 It is, therefore, important to know whether or not reduction in the ionized fraction of the serum calcium alters the action of digitalis drugs. The effects of reduced calcium are being clarified by the use of chelating agents such as the salts of ethylene-diamine-tetra-acetic acid (EDTA) or versenate. The major chemical property of EDTA is the capacity to form poorly ionized combinations with polyvalent cations. Cation-chelate complexes fail to give the usual reactions ascribed to the metal ions. In the physiologic pH range EDTA combines preferentially with calcium. The resulting complex forms stoichiometrically, each gram of EDTA binding 106 mg. of calcium. Following parenteral administration the unaltered complex is excreted almost quantitatively in the urine by means of filtration and active tubu-

Electrocardiographic Effects of Lowering Serum Calcium with EDTA: It has been reported that administration of EDTA controls electrocardiographic manifestations of digitalis overdosage in both animals and man.49-53 The antiarrhythmic action of EDTA is, however nonspecific and it will abolish ectopic mechanism regardless of etiology. As an antiarrhythmic agent, EDTA is generally less effective than potassium.58

In recent studies in dogs it was found that a sustained reduction in serum calcium with EDTA resulted in a doubling of the dose of acetyl strophanthidin required to produce ventricular arrhythmias.54 The electrocardiographic changes resulting from digitalization were those which characterize potassium intoxication rather than digitalis overdosage. Peaking of the T waves, P-R interval prolongation, A-V block, atrial arrest and intraventricular block were among the changes. These hyper-kalemic electrocardiographic changes were not associated with an abnormal elevation in the serum potassium. Administration of EDTA in dogs receiving potassium has been reported to precipitate fatal potassium poisoning. 48

Hypotensive Effects of Hypocalcemia: the ionized calcium is lowered by means of a rapid infusion of EDTA, the cardiac output is reduced, hypotension develops and, if no corrective measures are undertaken, the animal will die in shock. Except for prolongation of the Q-T interval, the electrocardiographic pattern remains essentially unaltered. In effect, death results from failure of the heart to act as a pump. When digitalis is given together with EDTA, no such deleterious sequelae ensue.54 It may be that some instances of hypotension in the surgical patient are due to hypocalcemia. Such hypotension would not respond to administration of pressor amines or to fluid supplementation, since these measures would merely overload a hypodynamic myocardium. In view of the findings in the animal, calcium infusion or digitalization may be the more appropriate and rewarding therapeutic approach.

MAGNESIUM AND DIGITALIS

The absence of a simple and precise analytic method for the determination of magnesium has handicapped study of the action of this ion in the animal's electrolyte economy. 55 Magnesium plays a key role as an activator of numerous enzymes including the catalytic reactions concerned with the transfer of phosphate groups involving adenosine triphosphate and adenosine diphosphate. The adult human being has a total of about 25 gm. of magnesium or about 43 mg. per kg. of fat-free tissue. Like potassium it is predominantly intracellular in location. In the normal person the serum concentration ranges from 1.4 to 2.5 mEq. per L. About 35 per cent is protein-bound. As is true with calcium, the degree of binding varies with the pH. Studies with radioactive isotopes indicate that heart muscle exhibits a particular avidity for magnesium.56 When Mg28 is given to animals, the concentration within heart muscle is three to ten times

greater than in skeletal muscle over a fortyeight-hour period of observation. The distribution in the heart is not homogeneous; the greatest concentration of radioactivity is in the interventricular septum, followed by the left ventricle and then the right ventricle.⁵⁷ The great affinity of the myocardium for magnesium may reflect the dependence of heart muscle on continuous enzymatic release of oxidative energy. Indeed, mitochondria of the heart muscle are especially sensitive to magnesium deprivation. In magnesium-deficient rats, mitochondria of the heart exhibit an uncoupling of oxidative phosphorylation. Tissues other than the heart are less sensitive to magnesium deficit.58

Effects of Magnesium Administration: Abnormalities in cardiac function resulting directly from endogenous changes in magnesium have not been clearly established either in man or in experimental animals. The level of magnesium in the perfusate of an isolated papillary muscle may be altered from zero to 15 mM per L., without significantly affecting the form of the resting or action potential.9 In the intact organism magnesium administration results in cardiovascular changes. Most important is the fall in blood pressure. 59 High serum concentrations ranging from 5 to 15 mEq. per L. cause bradycardia, P-R interval prolongation, intraventricular block and eventually, cardiac arrest.60-62 The depressant action of magnesium is not mediated by the vagus nerve, since it persists after vagotomy or atropinization. 60,62,63 Electrocardiographic effects consisting of P-R interval prolongation and intraventricular block have been reported in uremic patients with hypermagnesemia.64

Magnesium exerts an antiarrhythmic effect on the human heart. This is probably due to a slowing of conduction and increase in the refractory period of heart muscle. The antiarrhythmic property of magnesium was first demonstrated in dramatic fashion in a patient with ventricular flutter due to an overdose of strophanthin.65 A normal mechanism was restored by injecting magnesium directly into the heart. Since this experience, magnesium has been employed therapeutically for the control of varied ectopic mechanisms. 66-69 The cardiac suppressive effect is rapid in onset but of short duration; rarely does it last more than eight minutes. In animals with digitalis intoxication the results have been inconsistent. Once digitalis toxicity is established, magnesium

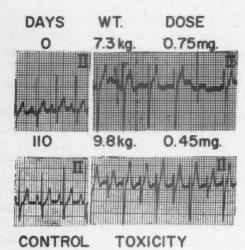


Fig. 2. Typical findings in puppies maintained on a magnesium-free diet. In this instance after 110 days without magnesium, the dose of acetyl strophanthidin necessary to produce ventricular tachycardia is reduced from 0.75 mg. (upper strip) to 0.45 mg. (lower strip). During this interval the animal's growth resulted in a weight gain of 2.5 kg.

administration will abolish the arrhythmia. However, when digitalization is accomplished during magnesium infusion, no protective effect is noted against toxic or lethal doses of digoxin. The divergence of results may be due to the fact that higher serum magnesium levels are required for control of arrhythmias than can be maintained for any duration without compromise of cardiovascular function. The transient action and the occasional undesirable side effects limit the use of magnesium for the the control of digitalis intoxication.

Effects of Magnesium Deficiency: No studies have been reported on the effects of magnesium deficiency on digitalis action. Low serum magnesium values have been noted in surgical patients maintained on parenteral magnesiumfree fluids or subjected to prolonged nasogastric suction.71-75 The magnesium content of gastric fluid ranges from 10 to 20 mEq. per L.; thus, large amounts may be removed through gastric intubation.76 A reduction in blood magnesium concentration has also been found in some patients with congestive heart failure, especially those on active diuretic programs. 72 A significant urinary loss of magnesium often follows a mercurial-induced diuresis. Some surgical patients with heart disease are therefore depleted of magnesium.

In studies currently in progress,⁷⁷ it has been found that magnesium deficiency sensitizes the heart to digitalis; a progressive reduction in the dose of digitalis necessary for the production

of ventricular tachycardia occurs in dogs that are made chronically deficient by dietary means (Fig. 2). The ventricular arrhythmias are multiform and usually persist for a longer duration than in control animals. In effect, the response to digitalis is as though the animals had been depleted of potassium. In this connection it is of interest that when rats are made magnesium deficient, a decline in content of potassium in skeletal muscle occurs. Magnesium deficiency may therefore play a role in the altered sensitivity to digitalis observed in some cardiac patients undergoing surgery.

EFFECTS OF DIGITALIS ON ELECTROLYTE DISTRIBUTION AND METABOLISM

Thus far, the effects of electrolyte changes on digitalis action have been considered. Of equal importance is the profound influence of these glycosides on the electrolyte distribution within the body. This was first suggested in the studies of the renal action of digitalis. In 1930, Gremels⁷⁹ observed a diuresis and chloruresis when digitalis-like drugs were administered to the isolated heart-lung-kidney preparation. This was true even when digitalis depressed renal blood flow. Gremels postulated that digitalis acted directly on the kidney tubules. Injection of digitalis glycosides into one renal artery, in a dose insufficient to alter cardiac hemodynamics, has been reported to produce a unilateral natriuresis and diuresis.81 In human beings without heart disease, digoxin promotes the excretion of sodium and chloride.82

Digitalis and Myocardial Electrolytes: Extensive studies have been carried out on the effect of digitalis on the electrolyte composition of heart muscle. There is general agreement that toxic doses promote loss of potassium in the heart muscle in such diverse species as the dog, rabbit, cat, rat, turtle and embryonic duck heart.83-89 Results have been inconsistent when less than toxic doses were employed. The entire range of possibilities from no change to increase or decrease in myocardial potassium has been observed.84-86,89,90 A loss of potassium in cardiac muscle has been found during acute digitalization of animals. 91,92 The transfer of electrolytes of the heart muscle was followed by determination of coronary sinusarterial gradients. These exchanges begin with the inception of drug action. A similar effect has been noted during chronic digitalization. Daily administration of 0.2 to 0.4 mg.

digitoxin to dogs partially inhibited potassium influx into the heart muscle with a reduction of intracellular potassium by 15 per cent. So In similar studies on patients with heart failure the coronary arteriovenous difference of potassium remained unchanged when Cedilanid was the digitalizing glycoside. However, when the ultrarapid-acting agent acetyl strophanthidin was employed, a net myocardial loss of potassium without a gain in sodium was observed. So

Digitalis and Tissue Electrolytes: Digitalis also affects electrolyte transport in tissues other than the heart. The earliest investigations concerned skeletal muscle.96,97 Resting sartorius muscle suspended in a medium containing strophanthin loses potassium.98 The cardiac glycosides also inhibit the uptake of potassium and promote a gain in sodium in cold-stored red cells.99-103 All glycosides tested act similarly to inhibit the active phase of cation transport. Ouabain in low concentration (10-6 mM per L.) produces 75 to 80 per cent inhibition of red cell potassium influx with little effect on potassium efflux. At this concentration, ouabain does not impair phosphate and carbohydrate metabolism within the red cell.104 Strophanthin has also been shown105 to reduce the electrical potential across the wall of the large intestine and partially inhibit the active transport of sodium and bicarbonate ions. The digitalis glycosides therefore alter electrolyte transport in many diverse tissues.

Digitalis and Serum Electrolytes: Do digitalis drugs significantly affect electrolyte transport in the intact organism? This problem was studied106 in forty dogs during 110 acute digitalizations to an end point of ventricular tachycardia. Digitalization caused a consistent and statistically significant rise in the serum potassium concentration averaging 0.64 mEq. per L., and a fall in the serum sodium concentration averaging 3.1 mEq. per L. No change was induced in the total serum calcium concentration (Table II). The increase in potassium occurred in 102 of 110 digitalizations (93 per cent); in six there was no change and in the remaining two, potassium was reduced. The decrease in sodium occurred in ninety-one of the 110 digitalizations (83 per cent); in ten there was no change and in nine there was an increase. In any one animal the magnitude of the sodium and potassium shifts appeared unrelated. The earliest change in cation was noted at the inception of digitalization and pre-

TABLE II

Changes in Serum Electrolyte Concentrations During 110 Digitalizations of Forty Dogs to the End Point of Ventricular Tachycardia

Degree of Digitalization	Na+ (mEq./L.)	K+ (mEq./L.)	Ca++ (mEq./L.)
Control Ventricular	148.0	4.53	5.30
tachycardia Ventricular	144.9	5.17	5.40
fibrillation*	136.7	6.33	

Note: At the emergence of toxicity a significant rise in potassium and a fall in sodium occurs while the calcium remains unchanged (p less than 0.01). The alterations in potassium and sodium are further accentuated when toxicity has reached the point of ventricular fibrillation (p less than 0.01).

* Ten animals.

ceded alterations in the electrocardiogram. The electrolyte shift assumed statistical significance when 60 per cent of the toxic dose had been given. When digitalization was carried to ventricular fibrillation, the rise in potassium averaged 1.8 mEq. per L., while the fall in sodium was 11.3 mEq. per L. These changes in electrolytes occur when the animal is artificially ventilated with room air as well as when oxygen is employed.

Electrocardiographic Effects of Digitalis and Electrolyte Shifts: The shift in potassium attending digitalization is of sufficient dimension to affect the T wave in the electrocardiogram. In 10 per cent of patients receiving maintenance digitalis therapy, a late peaking of the T wave has been observed.107 In 42 per cent of dogs a similar alteration in contour of the T wave developed during digitalization with acetyl strophanthidin. When the serum potassium concentration was increased to the level observed during acute digitalization, no similar peaking of the T wave occurred. It has been commented earlier that when digitalization was carried out while the serum potassium was fixed at an elevated level, the electrocardiographic changes were those of advancing potassium poisoning. Similarly, when calcium was lowered during digitalization, the pattern was that of hyperkalemia, although the concomitant rise in serum potassium was of itself insufficient to account for this change. In each of these instances, a digitalis-induced loss of myocardial potassium could be the underlying factor. This would suggest that the hyper-

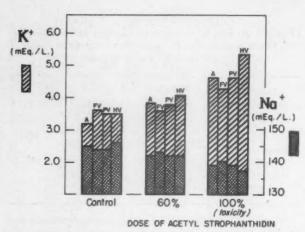


Fig. 3. Comparison of plasma concentrations of potassium and sodium in the dog at four simultaneously determined sites during peripheral digitalization. It is evident that the potassium concentration in the hepatic veins is higher than that of any of the other areas studied, as digitalis acts. The effect is most marked at the toxic end-point of ventricular tachycardia. A = artery; FV = femoral vein; PV = portal vein; HV = hepatic vein.

kalemic electrocardiographic pattern is the resultant of a transcellular potassium concentration gradient which is amplified by a reduction of the ionized fraction of serum calcium.

Release of Potassium by the Liver Following Digitalization: Which tissues or organs are responsible for the increase in serum potassium occurring during digitalization? The heart seems an unlikely source. First, since the cardiac blood flow constitutes only about 5 per cent of the total cardiac output, the observed rise in coronary sinus potassium is not adequate to increase the arterial serum potassium concentration to the levels observed during digitaliza-Second, the increase in arterial blood potassium level in some animals was in excess of the total calculated content of potassium in heart muscle. Efflux from skeletal muscle is probably not a factor. Study of potassium and sodium A-V gradients across skeletal muscle does not indicate any changes that could account for the observed deviations in arterial concentration.

Simultaneous sampling of blood from the hepatic vein, portal vein, splenic artery and femoral vein¹⁰⁸ during digitalization of chronically prepared animals has partially clarified the nature of the electrolyte drifts. ¹⁰⁹ Eleven such animals (referred to as "liver-cannulated dogs") were fractionally digitalized with acetyl strophanthidin. In these dogs the mean arterial potassium increment was 0.7 mEq. per L., while the decrement in sodium was 3 mEq.

per L. Thus, the electrolyte shifts were of the same magnitude as encountered in the larger control populations. In the liver-cannulated dog, both at mid-digitalization and at toxicity, the hepatic venous potassium exceeded concentrations at all other sites tested. At the onset of ventricular tachycardia, the hepatic venous potassium concentration was 0.8 mEq. per L. greater than the simultaneously determined arterial level, while the sodium value was 2.0 mEq. per L. less (Fig. 3). These differences were statistically significant. The electrolyte shifts across the liver were accentuated when the acetyl strophanthidin was given into the portal vein. In such transhepatic digitalization the hepatic venous potassium exceeded the simultaneous portal venous and arterial levels by 1.4 mEq. per L., while the sodium was 4 mEq. per L. less. No release of hepatic glycogen in the form of sugar accompanied the discharge of potassium from the liver. No significant shifts in hydrogen ion were detected. Thus it appears that the liver plays a major role in the electrolyte deviations attending digitalization.109

Reduced Uptake of Potassium by Skeletal Muscle: During this series of experiments the blood flow from the liver was measured in two of the animals, permitting an estimation of the maximum potassium release by the liver during digitalization. The quantity released by the liver did not exceed 10 mEq. When this amount of potassium is administered intravenously to nondigitalized animals, no significant increase in arterial concentration occurs. It is therefore apparent that hepatic release of potassium does not explain the digitalis-induced rise in arterial concentration. Some additional mechanism must be postulated. To explore this problem further, the shift in electrolytes in arterial and peripheral venous blood was determined during potassium infusion and concomitant digitalization. A prominent gradient of potassium was found between peripheral artery and vein, clearly indicating the role of skeletal muscle in the distribution of this cation. When 30 to 40 mEq. of potassium was infused in one hour, in the absence of digitalis, about 90 per cent of the administered load was taken up by the tissues (Table III). The rate of potassium uptake was strikingly reduced when the same quantities of potassium were infused during digitalization. This was demonstrated by the marked increase in arterial concentration and by the decrease or near disappearance of the

TABLE III

Effect of Digitalization on the Arterial Potassium Concentration and Its Uptake by the Tissues in Ten Dogs Infused with Potassium at a Mean Rate of 35 mEq./hr.

Digitalis	Rate of Arterial K + Increase (mEq./min.)	% of Infused K + Taken Up By Tissues
0 +	0.055 (0.029-0.10)* 0.283 (0.125-0.470)*	91.1 (85–95)* 63.7 (7.0–88.0)*

Note: The presence of digitalis reduces the percentage uptake by the tissues and results in a sharp increase in arterial concentration.

* Range.

A-V gradient across skeletal muscle. If digitalization was not halted, the animals succumbed from hyperkalemia.

Two mechanisms are therefore operating in the rise of serum potassium during digitalization. First, there is a generalized release of potassium from many tissues, with the liver making a major contribution. Second, uptake of the released potassium by skeletal muscle is inhibited. These mechanisms are also active in the digitalized patient. For this reason the treatment of digitalis intoxication with potassium, in the presence of advanced degrees of heart failure, or in the elderly patient, is not without hazard. With these facts in mind we can now turn to some clinical problems encountered in the digitalized surgical patient.

PREOPERATIVE CARE OF THE CARDIAC PATIENT

Improper preoperative management of the patient with heart disease frequently sets the stage for the problems encountered during operation and in the immediate postoperative period. The prerequisites of proper preparation include psychologic and physical rest, avoidance of excessive medication, an edemafree state and optimal digitalization.

The anxiety that inevitably precedes surgery in all patients may complicate the achievement and maintenance of compensation in the patient with heart failure. On one hand, a rapid heart rate may be brought on by anxiety and result in the administration of an overdose of digitalis. On the other hand, emotional stress will occasionally provoke ectopic beats, anorexia or vomiting, interpreted as hallmarks of digitalis

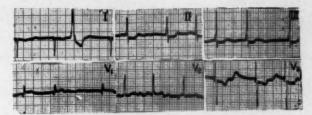


Fig. 4. Case 1. Slow ventricular response and ventricular ectopic beats during atrial fibrillation, suggestive of digitalis overdosage.

intoxication, and lead to discontinuance of this drug. Emotional tension further interferes with the mobilization of edema fluid by diuretics. Tranquilizing medications alone do not dissipate such anxiety and are not free of side effects. The patient's peace of mind is best secured not by drugs, but by the reassurance provided by a sensitive and understanding physician and by confidence in the surgeon.

The cardiac patient is frequently encumbered by an array of medications. Most are usually of little value and merely serve to complicate effective therapy. Some of the drugs in current use predispose to gastrointestinal upset which may be mistakenly interpreted as evidence of digitalis overdosage. Some provoke ventricular ectopic beats. For example, reserpine, which has been recommended for the preoperative preparation of the cardiac patient because of its sedative and bradycardic action, predisposes to hypotension during anesthesia¹¹⁰ and sensitizes the heart to the toxic action of digitalis.¹¹¹ The surgical patient should be given only necessary medications.

Preoperative Diuretic Administration: Preoperative preparation includes the mobilization of edema, the presence of which may seriously jeopardize pulmonary, hepatic and renal function during operation and in the days immediately following. However, the overenthusiastic use of the potent diuretics currently available results in profound electrolyte derangements. In the seriously ill cardiac patient digitalisintoxication attends such derangement. Several illustrative cases are presented.

Case 1.* A fifty-two year old woman (B. K., PBBH 9K551) in advanced right- and left-sided heart failure was being prepared for reoperation for recurrent mitral stenosis. Her program prior to hospitalization included rigid sodium restriction, weekly mercurial injections and digoxin in a dose of 0.25 and 0.5 mg. on alternate days. On admission, the

* Reported through the courtesy of Dr. Samuel A. Levine.

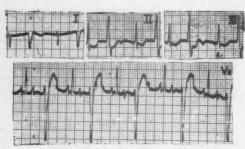


Fig. 5. Case 1. Four hours after mercurial-induced diuresis advanced digitalis intoxication emerges, characterized by ventricular bigeminy and paroxysmal atrial tachycardia with block at an atrial rate of 220 per minute.

rhythm was atrial fibrillation at a ventricular rate of 80 per minute with occasional ventricular ectopic beats (Fig. 4). Shortly after entry, having been prepared for two days with 3 gm. each of NH₄Cl and KCl, she was given 2 ml. of Thiomerin® followed in one hour by 0.5 gm. aminophylline administered intravenously. Within four hours her urinary output was 2,000 ml. and long runs of bigeminy superimposed on paroxysmal atrial tachycardia with block appeared (Fig. 5). The patient was now critically ill and was unable to undergo surgery. Two additional weeks of more judicious preparation were necessary before operation could be carried out.

Comment: In the presence of ventricular ectopic beats administration of diuretics is contraindicated until it is established that digitalis is not a causative factor. Serious arrhythmia may otherwise follow as illustrated in this patient. In the presence of advanced cardiac decompensation, especially with congestive hepatomegaly, the diuretic program must be instituted gradually and carried out over a prolonged period of time. Potassium supplementation does not afford protection

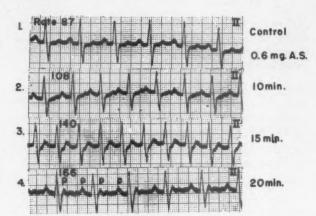


Fig. 7. Case 2. An acetyl strophanthidin tolerance test carried out one day after the record shown in Figure 6 was taken provoked an arrhythmia similar to that encountered during induction of anesthesia (compare strip 3 with strip 2, Figure 6). Without additional drug administration the arrhythmia progressed to paroxysmal atrial tachycardia with atrioventricular block (strip 4). This indicated that the initial arrhythmia observed during anesthesia was a manifestation of digitalis intoxication.

against digitalis intoxication unless it fully replaces the urinary losses. Optimal digitalization connotes both rate control and myocardial compensation achieved with a dose level that does not bring the patient to the brink of toxicity.⁴

Digitalis Overdosage Preoperatively: A patient may be given too much digitalis before operation, even though overt intoxication is not evident. Induction of anesthesia or the operation itself may then precipitate rapid heart action. At times this poses a nearly insoluble problem as to whether the tachycardia is the result of an excessive or of an inadequate amount of digitalis.

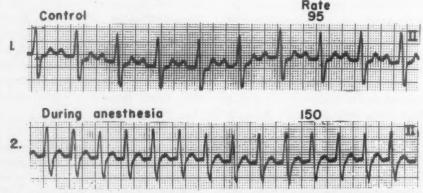


Fig. 6. Case 2. Supraventricular tachycardia occurring during induction of anesthesia in patient maintained on a large dose of digitalis. Is this to be interpreted as sinus tachycardia, paroxysmal atrial tachycardia or paroxysmal atrial tachycardia with block?

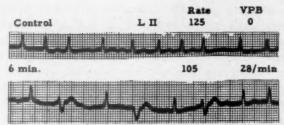


Fig. 8. Case 3. Administration of 0.15 mg. acetyl strophanthidin resulted in the appearance of ventricular premature beats within one minute which progressed within six minutes to bigeminy (lower strip). This persisted for ten minutes. The marked sensitivity to digitalis suspected on clinical evidence was confirmed by this test

CASE 2.* A forty-four year old man (W. G., PBBH 3K631) with severe aortic insufficiency was being prepared for operation. In the week prior to the procedure he lost 4 kg. as a result of increasing the dosage of digitalis leaf to 0.2 gm. daily. He received no mercurial diuretics. Serum electrolyte levels were within normal range.

During induction of anesthesia there was an abrupt increase in heart rate to 140 per minute. P waves could not be identified (Fig. 6). The operation was postponed and the patient was returned to his room. Prompt reversion to normal sinus rhythm occurred without a change in medication.

In order to define the patient's digitalis status as well as to identify the arrhythmia, an acetyl strophanthidin tolerance test was carried out (Fig. 7). After receiving 0.6 mg. of this drug an arrhythmia similar to that seen during induction appeared and progressed to full-blown paroxysmal atrial tachycardia with atrioventricular block. This disorder was controlled with potassium chloride. Digitalis was thereupon discontinued. Two days later the surgical procedure was undertaken without a recurrence of any arrhythmia.

Comment: This patient exhibited no stigmas of digitalis overdosage, yet during induction of anesthesia a supraventricular tachycardia developed which later was proved to have been due to excessive digitalis. In effect the arrhythmia presented a pattern which may be viewed as a phase in the development of paroxysmal atrial tachycardia with atrioventricular block. This latter disorder usually connotes far-advanced digitalis intoxication.2 The usual practice is to administer additional digitalis to control such an atrial arrhythmia. The course of this patient emphasizes the critical importance of the proper analysis of such a supraventricular tachycardia in the digitalized surgical patient.

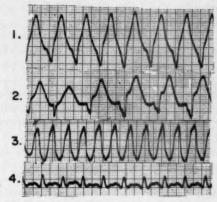


Fig. 9. Case 3. Part of a continuous electrocardiogram (Lead II) taken several days after that shown in Figure 8. This is characteristic of advanced potassium intoxication with associated acidosis and hyponatremia. There is nearly complete disorganization of the ventricular complex (Strips 1 to 3). This pattern returned virtually to normal in thirty minutes (strip 4) following the administration of 115 mEq. of sodium as the lactate and bicarbonate salts. Serum potassium, 7.8 mEq. per L.; serum sodium, 125 mEq. per L.; serum chloride, 100 mEq. per L.; serum carbon dioxide, 12 mEq. per L.

Digitalis Toxicity and Hyperkalemia in Advanced Congestive Failure: Another preoperative problem is presented by the patient who is in advanced congestive failure with serious derangement of the liver and in whom it is almost impossible to determine whether the amount of digitalis given is adequate for therapeutic effect or represents an overdosage. Occasionally one sees the clinical counterpart of the experiment in which digitalis intoxication leads to severe hyperkalemia. We have actually observed several patients with digitalis poisoning who died from hyperkalemia as a result of small oral doses of potassium.

Case 3. A fifty-three year old woman (V. S., PBBH R1226) was admitted for mitral valvuloplasty. She had suffered from the effects of mitral valve stenosis for eleven years. On examination she manifested the typical features of tight mitral stenosis with atrial fibrillation, cyanosis, a large pulsating liver and advanced anasarca. A major problem in her management had been fluctuations between underdigitalization and toxicity. The ventricular rate was consistently above 100 per minute. Digoxin in a dose of 0.25 mg. on alternate days evoked bigeminal rhythm. It was therefore discontinued. After digitalis was withdrawn for fifteen days an acetyl strophanthidin tolerance test was carried out (Fig. 8). After an initial dose of 0.15 mg. (approximately one-tenth of a digitalizing dose) bigeminy appeared with a slowing of ventricular rate from 125 to 105 per minute.

In order to achieve rate control without toxicity prior to operation, potassium supplementation was

^{*} Reported through the courtesy of Dr. Dwight E. Harken.

increased from 3 to 5 gm. daily and 1 gm. procaine amide was added. Gitaligin® was given in a dose of 0.75 mg. a day. At this time the serum sodium and potassium and blood urea nitrogen levels were normal. One day later the ventricular rate had slowed to 80 to 90 per minute with an occasional ventricular premature beat. On the second day the rate again accelerated to 130 per minute and became regular. There was atrial standstill with marked intraventricular block and prominent peaking of the T waves (Fig. 9). The blood pressure was unobtainable and the patient appeared moribund. The serum electrolytes exhibited the following values: potassium 7.8 mEq. per L.; sodium 125 mEq. per L.; carbon dioxide 12 mEq. per L.; chloride 100 mEq. per L.; and the blood urea nitrogen was 14 mg. per 100 ml. Intravenous administration of sodium lactate and sodium bicarbonate caused transient reversion of the arrhythmia to normal intraventricular conduction and atrial flutter (Fig. 9). This improvement was maintained only so long as the saline infusion was continued. Pulmonary edema prevented further salt loading. Clinical deterioration recurred and was followed rapidly by cardiac standstill and death.

Comment: On prior occasions during the terminal hospitalization, this patient tolerated similar doses of potassium without deleterious effect. The potassium prevented the development of digitalis-induced ventricular ectopic beats and permitted slowing of the ventricular rate. As in the experimental animal, however, larger doses of digitalis, when given in conjunction with potassium, interfered with the cellular uptake of this cation and resulted in hyperkalemia and death.

Recent studies confirm the concept that digitalization impairs the uptake of administered potassium. 112 Observation of similar phenomena in man has led some to the conclusion that potassium may potentiate digitalis toxicity.113-115 However, the results are most consistent with digitalis enhancement of the effect of potassium on the heart due to interference with the intracellular transport of this cation. Under what conditions does digitalis impede the transcellular migration of potassium? It should be noted that digitalis does not block completely but merely reduces the rate of the cellular ingress of potassium. It follows that in the normal animal at a slow rate of potassium administration, digitalization to toxicity will not result in hyperkalemia. Similarly, when potassium is given to patients either orally or intravenously at a rate of less than 0.5 mEq. per minute, no interference in uptake is demonstrable. Since the efficacy of potassium in

combating digitalis intoxication, other than transiently, is a function of the amount of potassium given rather than the rate of its administration, potassium can be safely given to counteract digitalis intoxication. The exceptions to this generalization are patients with far-advanced heart failure or renal disease, and the very old.

It has been our experience that in a patient who manifests digitalis intoxication before the full therapeutic action of this drug progressive hyperkalemia is especially prone to develop. The presence of dilutional hyponatremia as well as deranged function of the liver increases the possibility of digitalis-induced potassium poisoning. Such patients present problems that are almost insuperable in preparation for operation. The operative trauma, which may itself lead to hyperkalemia, further predisposes to such digitalis-induced electrolyte derangements.

THE POSTOPERATIVE PERIOD

In the postoperative period problems related to the use of digitalis may be divided into three major categories: (1) rapid heart action; (2) congestive failure; and (3) electrolyte derangements.

RAPID HEART ACTION

Paroxysmal Arrhythmias in the Undigitalized Patient: The development of a paroxysmal arrhythmia in the postoperative patient constitutes an emergency that demands immediate determination of the nature of the disorder and prompt measures for its control. This is especially true in the elderly cardiac patient in whom rapid heart action gravely compromises cardiac output, leading to hypotension and a critical reduction of coronary artery perfusion. In such patients even brief periods of hypotension are hazardous since coronary flow is already reduced by atheromatous disease.

The most serious paroxysmal disorder is ventricular tachycardia. Even at slow rates the aberrant activation of the myocardium impairs contractility and reduces cardiac output. The arrhythmia frequently is caused by myocardial infarction which may have resulted from hypotension in the intraoperative period. Recognition of the myocardial injury is made difficult by the absence of implicating symptoms. Treatment consists of the administration of procaine amide or quinidine. Digitalis is generally not indicated unless the antiarrhythmic measures prove unavailing and the

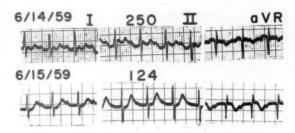


Fig. 10. Case 4. Atrial flutter. Three days after radical pneumonectomy atrial flutter appeared with a ventricular rate of 160 to 200 per minute. Administration of large amounts of digitalis were required to slow the ventricular response (upper strip) although the atrial rate remained at 250 per minute. On the following day (lower strip), the ventricular rate is unchanged but P wave morphology is altered, suggesting a different atrial mechanism.

patient exhibits evidence of congestive heart failure.

The simplest arrhythmia that may occur in the postoperative period is atrial or nodal tachycardia.80 Carotid sinus massage or other vagusstimulating maneuvers may be all that is required. If these fail, rapid digitalization is indicated. The agents to be employed are the rapid-acting digitalis derivatives such as acetyl strophanthidin, ouabain, digoxin or Cedilanid. The choice of any single preparation depends on the urgency of the situation and the physician's experience with the drug. Carotid sinus massage should be employed before each additional increment of a digitalis drug is administered since this will facilitate reversion with lesser amounts of the drug than would otherwise be required.

Atrial fibrillation and atrial flutter are among the commonest forms of paroxysmal rapid heart action encountered in the postoperative period. These disorders occur in 40 to 50 per cent of patients undergoing mitral valvuloplasty who are in normal sinus rhythm prior to operation. The incidence after pulmonary resection is variously reported as 20 to 30 per cent, rising to as high as 50 per cent when intrapericardial division of the hilar vessels is carried out.117 The major aim of therapy is to reduce the ventricular rate by impairing A-V conduction. These arrhythmias generally revert spontaneously either immediately or within a few days after the ventricular rate has been controlled. Administration of drugs such as acetyl strophanthidin is contraindicated since the dissipation of effect within one to two hours is followed by reacceleration of the ventricular rate. The use of Prostigmin, which transiently slows the

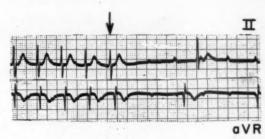


Fig. 11. Case 4. Effect of carotid sinus massage. During the tracing shown in the lower part of Figure 10 carotid sinus massage was begun (arrow). This resulted in complete A-V block without a change in the atrial rate, identifying the mechanism as paroxysmal atrial tachycardia with atrioventricular block. This arrhythmia is most frequently the result of digitalis intoxication.

heart, offers no advantage over rapid digitalization. Prostigmin should be avoided in patients with a bronchospastic history.

The development of paroxysmal rapid heart action in the postoperative patient requires, in addition to identification of the arrhythmia, a careful analysis of the precipitating causes. In some the arrhythmia may be the cause and in others the consequence of hypotension. Reduction in blood volume is but one of many causes of hypotension occurring after operation.

CASE 4. A sixty-six year old man (W. H., PBBH 7N124) underwent a right pneumonectomy with intrapericardial division of the hilar vessels for the removal of a carcinoma which invaded the lower trachea. His course was benign until the third postoperative day when the cardiac rate accelerated to 160 to 200 per minute. At this rate the systolic blood pressure ranged between 70 and 80 mm. Hg. An intravenous drip of Neosynephrine® restored the blood pressure to 110 mm. Hg but did not result in slowing of the pulse. The electrocardiogram showed paroxysms of atrial flutter alternating with atrial fibrillation. There was, in addition, S-T segment elevation in leads II, III, aVF and V6 which was at first ascribed to the initial changes of an acute myocardial infarction. In view of the known sensitivity to digitalis in the presence of myocardial infarction, he was given only 0.75 mg. of digoxin. This failed to control the rate. Since his condition was deteriorating, rapid digitalization was carried out with ouabain, employing a total dose of 1 mg. over the next four hours. The rate then slowed to 120 per minute and the changes suggesting myocardial infarction abated, leaving only evidence of pericarditis.

Despite the reduction of heart rate the blood pressure could not be maintained without Neosynephrine. An x-ray film of the chest showed fluid up to the level of the first rib anteriorly on the operated side, indicating rapid exudation of blood and plasma from the extensive mediastinal dissection. It was estimated that a deficit of 3,000 ml. of fluid existed.

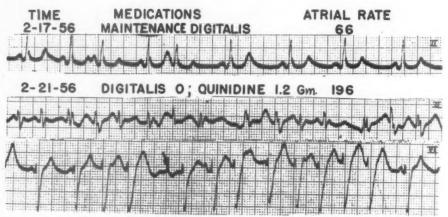


Fig. 12. Case 5. Atrial and nodal premature beats appeared on the first day after mitral valvuloplasty (upper strip). Digitalis was omitted and quinidine was administered in a dose of 1.2 gm. daily. On the fifth postoperative day a more complex arrhythmia developed (lower strips). Is this arrhythmia the result of digitalis intoxication or can it be controlled by digitalis supplementation?

This was replaced with blood, plasma and saline while the venous pressure was continuously monitored. Thereafter, blood pressure was maintained without administration of vasopressor agents. The patient, however, remained in atrial flutter with 2:1 A-V block and a ventricular rate of 120 per minute, and large additional doses of digoxin were necessary to control it. On the sixth postoperative day the atrial pacemaker altered without a change in heart rate (Fig. 10). Carotid sinus massage indicated the mechanism to be paroxysmal atrial tachycardia with block, clear evidence of digitalis intoxication (Fig. 11). Digitalis was therefore stopped and potassium chloride and procaine amide given with restoration of normal sinus rhythm. Recovery thereafter was uneventful.

Comment: In this patient it seemed likely that loss of blood and plasma into the pleural space produced an intravascular volume deficit with a reduction of cardiac output and hypotension. The ensuing compromise of coronary blood flow resulted in myocardial anoxia and may have predisposed to the atrial arrhythmia. Proper treatment necessitated reduction in ventricular rate, increase in blood pressure and restoration of intravascular volume. Large doses of digitalis were required to control the heart rate. Ouabain was an ideal agent in this particular situation. It permitted the administration of increments at thirty-minute intervals, the time required for the peak action of this drug. Had a slower acting glycoside been employed, several days might have been necessary to slow the rapid heart rate. Permanent myocardial damage and death might then have been the outcome.

Of further interest is the development of

digitalis intoxication in this patient on the sixth postoperative day. The night before, 1 mg. digoxin was required to control a ventricular rate of 150 per minute. The mechanism was still that of atrial flutter. The next morning the heart rate was 125 per minute. Superficial inspection of the electrocardiogram would have suggested that this patient was still in atrial flutter at a rate of 250 per minute with 2:1 A-V block. However, a definite change in morphology of the P-wave had occurred. Carotid sinus pressure divulged the true nature of the disorder as paroxysmal atrial tachycardia with atrioventricular block. Furthermore, the electrocardiogram was now consistent with the pattern of potassium deficiency. The serum potassium level, as expected, was within normal range. This patient had been experiencing a substantial postoperative diuresis.

When paroxysmal rapid heart action occurs in the postoperative period and is associated with hypotension, the first procedure must be the institution of measures for the maintenance of an adequate blood volume and blood pressure. The use of vasopressor agents not only restores coronary perfusion by increasing peripheral resistance but also may restore a normal sinus mechanism.118 After restoration of blood volume, other measures to correct hypotension are initiated, the arrhythmia is identified and the need for digitalis is determined. It should be emphasized that the early use of epinephrinelike drugs is justified in paroxysmal rapid heart action whereas in the gradually developing tachycardia and hypotension of oligemia, restoration of blood volume has first priority.

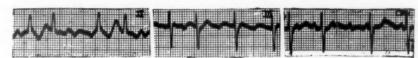


Fig. 13. Case 5. Administration of digoxin in a dose of 0.75 mg. intravenously promptly slowed the heart rate, reduced the ventricular aberration and revealed the underlying disorder to be that of atrial flutter, here associated with inadequate digitalization.

Paroxysmal Arrhythmias in the Digitalized Patient: In the digitalized patient the problem presented by the emergence of a paroxysmal arrhythmia is complicated by the possibility that the disordered mechanism may be due to digitalis. If the arrhythmia is clearly identified as atrial fibrillation or flutter, overdigitalization is unlikely. In such a case, if the ventricular rate is rapid, the dose of digitalis should be increased until adequate slowing is achieved. When the atrial mechanism cannot be identified or when the atrial rate is less than 250 per minute, it is difficult to be certain whether or not digitalis is implicated. This is especially a problem when the ventricular rate is rapid and aberration exists in ventricular conduction. It is then difficult to be certain whether the arrhythmia is due to atrial flutter, paroxysmal atrial tachycardia with atrioventricular block, nodal tachycardia or even ventricular tachycardia.

CASE 5. A fifty-two year old woman (M. J., PBBH B651) with combined hypertensive heart disease and mitral stenosis with left-sided heart failure, was on maintenance digitalis therapy. The day following mitral valvuloplasty she exhibited numerous atrial premature beats with aberration in ventricular conduction (Fig. 12, strip 1). She was in normal sinus rhythm preoperatively and was maintained on quinidine to prevent the occurrence of atrial fibrillation. During the next four days, digitalis was discontinued because of sinus bradycardia, sinus pauses, nodal escape and brief runs of nodal rhythm. Since multiple atrial premature beats may be premonitory of atrial fibrillation, quinidine administration was continued. A bizarre arrhythmia then emerged with an atrial rate of 196 per minute and ventricular complexes of variable contour and duration (Fig. 12, strips 2 and 3). This disorder was initially interpreted as paroxysmal atrial tachycardia with atrioventricular block with ventricular premature beats and paroxysmal ventricular tachycardia. The circumstances surrounding this case made atrial flutter, accompanied by aberrant ventricular conduction, the more likely disorder. Because of the patient's critical status she was given digoxin intravenously. The pulmonary edema which had developed cleared rapidly as the ventricular rate slowed. The background rhythm was now clearly atrial flutter (Fig. 13). Additional

doses of digoxin resulted in atrial fibrillation with a slow ventricular response.

Comment: In this patient the clinical impression of underdigitalization contradicted the electrocardiographic interpretation of digitalis intoxication. This emphasizes the pitfall of placing too much reliance on any one laboratory aid, the electrocardiogram being no exception. The pathogenesis of an arrhythmia cannot be decided on the basis of the electrocardiographic tracing except when taken in context with the total clinical situation. In this particular patient the abnormal rhythm developed four days after administration of digitalis had been discontinued and there had been no abnormal diuresis. In such an instance digitalis intoxication is unlikely. A number of facts suggested that the rhythm was atrial flutter modified by the administration of quinidine. First, in a patient in normal sinus rhythm undergoing mitral valve surgery, atrial flutter or fibrillation commonly occurs. Second, the appearance of ectopic atrial beats in such an instance augurs the development of one of these disorders. Third, the discontinuance of digoxin would permit a rapid ventricular response once atrial flutter or fibrillation developed. The vagolytic action of quinidine would further enhance A-V conduction and speed the heart rate. Finally, quinidine favors a slow atrial rate in flutter and predisposes to ventricular aberration.

In patients receiving both quinidine and digitalis the arrhythmias which develop in the post-operative period are usually variants of atrial flutter. Digitalis is generally the drug of choice in their management.

Sinus Tachycardia: Among the cardiovascular problems encountered in the postoperative period the appearance of sinus tachycardia is unquestionably the most common. When it occurs, the advisability of digitalization is invariably considered. It must be recognized, however, that such a tachycardia is a normal physiologic response to the multiple and interrelated stimuli of the surgical operation, including especially loss of blood, anoxia, hypercarbia, pain and fear.

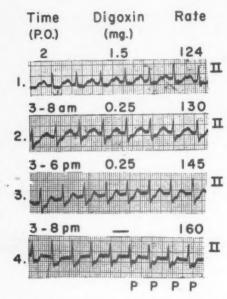


Fig. 14. Case 6. Digoxin, in a dose of 1.5 mg., given during the first two days after operation in an attempt to control the rate of sinus tachycardia, resulted in slight acceleration (strips 1 and 2). Additional increments of digoxin on the third postoperative day further accelerated the ventricular rate (strips 3 and 4) and resulted in the emergence of a complex rhythm consisting of a nodal tachycardia with A-V dissociation and partial synchronization of the two pacemakers. This is an example of advanced digitalis intoxication.

Sinus tachycardia is reflex in origin and primarily the result of a release of the inhibitory action of the vagus. It serves to augment the cardiac output in response to increased bodily requirements. The rate in sinus tachycardia is generally impervious to slowing by digitalis. This is fortunate for, as Wiggers states, "otherwise many a patient with serious heart disease would have been helped prematurely to his grave through kindly intentioned but misguided treatment." When large doses of digitalis are given in an attempt to slow a sinus tachycardia it may compromise cardiac output and also lead to serious toxicity.

In the digitalized postoperative patient the emergence of sinus tachycardia poses the question of the adequacy of digitalization. The fact that the rate is rapid is generally regarded as evidence of underdigitalization. In effect, however, the acceleration may not be caused by decompensation but by the operation. In this situation as well, additional administration of digitalis will not only prove ineffective but even dangerous.

Case 6. A forty-six year old man (J. P., PBBH 3N837) with rheumatic heart disease and predomi-

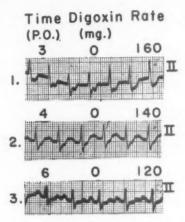


Fig. 15. Case 6. Omission of digitalis on the third postoperative day resulted in reversion to sinus tachycardia, with a gradual slowing of the heart rate to 120 per minute by the sixth day and an improvement in the patient's clinical condition.

nant mitral insufficiency, had been receiving 0.2 mg. digitoxin daily for three years. Because of bigeminal rhythm digitalis administration had been stopped for the week prior to surgery. He underwent an exploratory cardiotomy with placement of an extrinsic baffle for the alleviation of mitral insufficiency. Postoperatively he was slightly hypotensive and was given 500 ml. of blood and 250 ml. of plasma. This stabilized the blood pressure but resulted in a rise in venous pressure to 225 mm. water. He was febrile and had coarse rales at the bases of both lungs. A sinus tachycardia of 130 per minute was present. Digitalis administration was therefore resumed in the form of digoxin and over the course of the first three postoperative days he received 2 mg. After 1 mg. the venous pressure fell from 225 to 60 mm. water but the heart rate accelerated to 160 per minute. At this point the patient appeared critically ill. He was cold and clammy, extremely dyspneic and apprehensive. The usual subjective manifestations of digitalis overdosage were absent. The blood chemical analyses showed potassium 4.7 mEq. per L.; sodium 142 mEq. per L.; carbon dioxide 25 mEq. per L.; chloride 109 mEq. per L.; and blood urea nitrogen 49 mg. per 100 ml. An electrocardiogram revealed a transient double tachycardia with both sinus and nodal pacemakers (Fig. 14).

The arrhythmia was interpreted as due to digitalis intoxication. Digitalis was therefore omitted and procaine amide was administered. The arrhythmia promptly responded to these measures (Fig. 15). No digitalis was given on the following four days and the rate receded to 130 per minute. Venous pressure remained at 50 mm. water. The pulmonary process, which in retrospect must have been due to atelectasis and pneumonitis, had by this time cleared and the temperature had returned to normal. It was now thought that the persisting sinus tachycardia was due to left-sided heart failure. In the next five days

he was given a total of 5 mg. digoxin with a reduction in heart rate to 100 per minute and striking improvement in his clinical condition.

Comment: In the postoperative period the patient suffering from anoxia due to retained secretions, atelectasis, pneumonitis, reduced blood volume or pulmonary embolism will maintain a rapid heart rate irrespective of the amount of digitalis administered. Here, atelectasis appeared to be the cause. As emphasized previously, this tachycardia is a physiologic defense to maintain the cardiac output. Furthermore, increased sensitivity to the toxic effects of digitalis is often present, as illustrated in this patient. This susceptibility may well be due to depressed hepatic and renal function resulting from anoxemia and hypotension.

When increasing amounts of digitalis are given to slow a sinus tachycardia, a paradoxical response may occur, namely, the heart rate accelerates with each increment of digitalis. The sinus tachycardia is usually due to impaired pulmonary function. As the rate becomes more rapid the usual tendency is to increase the amount of digitalis rather than to stop it entirely. This course will frequently result in the patient's death. At postmortem examination the prominent anatomic process in the lung is apt to be held responsible for the final outcome. The role of digitalis is thus rarely recognized.

In the patient under discussion, in addition to an acceleration of rate, a disorder of rhythm appeared which is frequently the result of digitalis overdosage. That this was indeed the case was proved by the resolution of the arrhythmia and reduction of heart rate on omission of digitalis. It is not commonly recognized that a sinus tachycardia may be the result of digitalis intoxication, but such an arrhythmia is particularly prone to occur in the postoperative patient.

CONGESTIVE HEART FAILURE

In the postoperative period the recognition of left-sided heart failure before the onset of frank pulmonary edema is difficult. The heart rate may be accelerated for other reasons as previously described. A gallop is difficult to detect due to rapid rate and the masking effect of extracardiac sounds. Rales at the bases of the lungs are not easy to interpret in view of the common occurrence of atelectasis due to retained secretions. Dyspnea and orthopnea are often absent because of a dulled sensorium or the dis-

traction of coexisting pain. Tachypnea and cyanosis may be due to defects in pulmonary ventilation.

Enlargement of the liver develops rapidly in the child and constitutes a sensitive index of right-sided decompensation, but is of less value in the adult. Of the many indices of heart failure, the venous pressure is of particular usefulness in the postoperative patient. Constant monitoring of this modality is of utmost importance in any patient with heart disease who is receiving parenteral fluid therapy or transfusions of blood, plasma or albumin.

The complexity of this problem is clearly indicated by the course of one patient (Case 6). While the heart failure readily responded to administration of digitalis with a reduction of venous pressure to normal levels, the heart rate did not slow. When the pneumonitis had cleared and the heart rate was still rapid, left-sided decompensation appeared as a likely factor. This supposition was confirmed by the slowing that eventually occurred with large increments of digoxin. In the absence of pulmonary complications, blood volume deficit or significant pyrexia, the judicious use of digitalis may prove effective in slowing a sinus tachycardia, for in that case the tachycardia may be due to left ventricular failure.

ELECTROLYTE ABNORMALITIES

There are four characteristic families of metabolic alteration that may occur in the surgical patient and influence his state of digitalization. They are: (1) sodium-potassium shift; (2) oliguria and spontaneous diuresis; (3) acute acid-base changes; and (4) alteration in ionized calcium.

At the outset it must be emphasized that the relation between digitalis and electrolytes, as presently conceived, relates almost exclusively to effects on the excitability and conductivity properties of heart muscle. No knowledge exists concerning the effect of electrolyte alterations on the positive ionotropic effect of digitalis in the patient with heart failure.

Sodium-Potassium Shift: In its simplest form this consists in a fall in the serum sodium concentration to about 130 mEq. per L. and a rise in serum potassium concentration to about 5 mEq. per L. forty-eight hours after operation. 1,21,120 Its pathogenesis is not well understood but dilutional hypotonicity is almost unquestionably responsible for the hyponatremic aspect of this pattern. This disorder is markedly

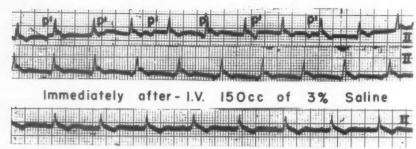


Fig. 16. Case 7. On the eighth day after an aortic valvuloplasty was performed, nodal tachycardia developed in the presence of a severe electrolyte derangement characterized as azotemia with hyperkalemia, hyponatremia, hypochloremia and a moderately severe acidosis (upper strip). The arrhythmia responded transiently to the administration of hypertonic saline (lower strip). This is an example of nodal tachycardia which was the result of a metabolic disorder rather than digitalis intoxication. Blood urea nitrogen, 105 mg.; serum sodium, 120 mEq. per L.; serum potassium, 6.7 mEq. per L.; serum chloride, 86 mEq. per L.; serum carbon dioxide, 14.2 mEq. per L.

aggravated by giving the patient too much water. It is somewhat alleviated by administration of sodium. Although the hyperkalemia which attends this alteration might be considered to be a result of the mobilization of potassium from cells, this simple explanation is not entirely adequate.

We prefer to identify as a separate entity the more severe sodium-potassium shift which is observed after surgical procedures carried out on patients with chronic diseases of the heart, liver, kidneys, or in chronic starvation. Prior to operation, such patients often manifest some dilutional hypotonicity with hyperkalemia. They will show high total body sodium values despite the low serum sodium concentration and there is a typical inversion of sodium and potassium body content and serum concentration. In such patients, when operated on, a marked accentuation of this defect develops, with a sodium concentration falling into the range of approximately 125 mEq. per L. and potassium rising to about 6.5 mEq. per L. This sequence is common in digitalized patients undergoing cardiac operations.

These electrolyte changes pose one of three problems in the digitalized patient. First, increased sensitivity to the toxic action of digitalis at the time of the post-traumatic electrolyte shift is sometimes observed despite the presence of a high serum potassium concentration. Second, the postoperative cardiac patient is predisposed to the interference with potassium transport induced by excessive digitalis. Finally, when of marked degree these electrolyte shifts often result in arrhythmias which simulate digitalis overdosage.

Case 7.* A fifty-three year old woman (A. I., PBBH D1998) had undergone a transaortic operation for calcific aortic stenosis. Prior to operation she was in normal sinus rhythm and had been digitalized for over two days with a total dose of 1.75 mg. digoxin. Her maintenance dose was 0.25 mg. daily. She had not been treated with diuretics or rigid sodium restriction. The major symptoms had been angina pectoris and mild fatigue on exertion. After the usual postoperative oliguria for two days (190 and 260 ml.) she sustained a diuresis of 1,975 ml. on the fourth postoperative day. Nevertheless, a progressive fall in sodium and rise in serum potassium concentration occurred. On the eighth postoperative day the blood urea nitrogen was 100 mg. per 100 ml.; sodium 120 mEq. per L.; potassium 6.7 mEq. per L.; carbon dioxide 14.2 mEq. per L.; and chloride 86 mEq. per L. The patient appeared critically ill with evidence of advanced right-sided heart failure. She had gained 4 kg. in weight. The electrocardiogram showed a nodal rhythm with variable degrees of forward block and A-V dissociation (Fig. 16), The question of digitalis intoxication was considered. However, the decision was that the patient was underdigitalized. She was given 300 ml. of 3 per cent saline and 1 mg. digoxin in the next twelve hours. This was followed by a dramatic improvement in her mental state. Normal sinus fhythm was transiently restored after the saline infusion. Increasing doses of digoxin were required to re-establish cardiac compensation. The restoration of a normal electrolyte pattern took place gradually over the next sixteen days, by which time she was again in normal sinus rhythm.

Comment: It has been pointed out that the cardiac patient may experience more marked degrees of dilutional hyponatremia after opera-

^{*} Reported through the courtesy of Dr. Dwight E. Harken.

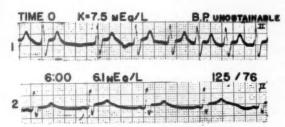


Fig. 17. Case 8. Twenty-four hours after aortic valvuloplasty was performed, shock and an arrhythmia characterized by atrial standstill, intraventricular block and irregular rhythm developed (strip 1). Hyperkalemia was present. Both the shock and the arrhythmia responded to reduction in serum potassium concentration (strip 2) achieved by the administration of hypertonic saline solution. Misinterpretation of this disorder as a manifestation of digitalis toxicity would prompt the clinician to administer potassium salts and thereby jeopardize survival.

tion than the non-cardiac patient. In this particular case inadequate restriction of water as well as inadequate digitalization with resultant aggravation of congestive heart failure appeared to be responsible for the severe electrolyte derangement. The arrhythmia that this patient manifested is most frequently caused by digitalis overdosage but it must be emphasized that no arrhythmia is ever pathognomonic of digitalis intoxication. The etiology of the disorder of rhythm can only be correctly deduced by a careful analysis of the total clinical situation. The fact that digitalization was carried out immediately prior to surgery in the presence of normal sinus rhythm did not permit the determination of the exact requirements of digitalis. The dose which the patient received is generally inadequate for full digitalization. The nodal tachycardia that developed was probably due to hyperkalemia and hyponatremia, since it cleared transiently after the administration of hypertonic saline. The irrationality of giving sodium to a patient with a high total body sodium is quite evident. Nonetheless, it is the plasma disturbance which provokes difficulties and this must be corrected. This patient's postoperative metabolic situation was an unusually exaggerated sodium-potassium inversion with a severe metabolic acidosis (carbon dioxide combining power 14.2 mEq.

The shifts in electrolytes are occasionally of such magnitude as to induce serious disorganization of heart rhythm. The question then arises as to whether the arrhythmia represents digitalis intoxication or is a reflection of the electrolyte situation.

Case 8.* A fifty-four year old man (M. E., PBBH F978) underwent operation for calcific aortic stenosis. During the procedure his blood pressure fell for a brief period to shock levels but was restored with an intra-arterial pressure transfusion of 1,000 ml. of bank blood. On the day of operation his condition remained good until 10 P.M. when his systolic blood pressure fell to 60 mm. Hg despite intravenous administration of norepinephrine. By the following morning blood pressure was unobtainable. The electrocardiogram now showed an absence of P waves, irregular ventricular rhythm, intraventricular block and slight peaking of the T waves (Fig. 17). Since he had received digitalis, procaine amide and quinidine, intoxication with any one of these agents seemed possible. From the electrocardiographic standpoint the disorder suggested potassium poisoning. Despite his precarious cardiac status, he was given 100 ml. of 3 per cent saline solution in the course of one hour followed by 700 ml. of 10 per cent glucose with 35 units of insulin. Improvement was rapid and his recovery from this point was continuous and uneventful. A blood sample drawn immediately before therapy revealed a serum potassium concentration of 7.5 mEq. per L. (Fig. 17).

Comment: In this patient the lack of atrial activity led to an initial diagnosis of nodal rhythm possibly due to digitalis. The administration of potassium was considered. However, digitalis intoxication was deemed unlikely for two reasons: first, digitalis does not cause intraventricular block; and second, the nodal arrhythmias produced by digitalis are generally regular; when irregular, atrial capture is usually evident. Development of hyperkalemia in this patient was traceable to oliguria and a small contribution of hemolyzed blood. Had potassium been administered under the erroneous impression of digitalis intoxication it would undoubtedly have resulted in death.

Oliguria and Diuresis: Several days of oliguria followed by a diuresis beginning on the second or third day after any trauma is a characteristic finding. This may be particularly marked if the trauma has been severe or has been attended by the formation of an area of inflammatory or sequestrational edema. The diuresis consists of both solute and water. The kidney is released from the antidiuretic effects of the immediate trauma and the tendency to conserve sodium is also lost. The osmolality of the urine tends to be fixed at about 700 mOsm. per L. and in many respects the response has the appearance of an osmotic diuresis.

^{*} Previously reported.12

When this occurs in a fully digitalized patient one of two things may happen. If the diuresis takes the form of water unloading without a marked change in body or serum potassium concentration, the digitalis status remains unaltered, and the patient's clinical status is remarkably improved. If the diuresis is associated with a fall in the body potassium, severe digitalis toxicity may develop and death may ensue as a result of a "normal" spontaneous postoperative diuresis. In such an instance it may never be certain that digitalis was implicated.

CASE 9. A seventy-five year old man (W. S., PBBH 8L425) underwent an uneventful esophagectomy for carcinoma of the esophagus. Prior to operation he lost 25 pounds. He was in normal sinus rhythm and had no clinical evidence of heart disease, but there was mild azotemia (blood urea nitrogen 40 mg. per 100 ml.) and hyperkalemia (5.5 mEq. per L.) before operation. He had marked oliguria for the first two postoperative days. His blood chemical analyses were: potassium 5.9 mEq. per L.; sodium 128 mEq. per L.; carbon dioxide 21 mEq. per L.; chloride 93 mEq. per L.; and blood urea nitrogen 35 mg. per 100 ml. On the third postoperative day a diuresis began and his urinary output rose to 1,790 ml. The next day his output was 2,125 ml. and a paroxysm of atrial fibrillation developed with a ventricular rate of 140 per minute. Digitalization was begun with 0.75 mg. digoxin. This was followed by prompt reversion to normal sinus rhythm. Thereafter, he received an additional 1.5 mg. digoxin over the next two days. The diuresis continued and on the sixth day, three hours after receiving 0.25 mg. digoxin orally, he became pulseless and died. The day before, his electrolytes had returned to normal values with a marked fall in potassium, although the blood urea nitrogen was still 80 mg. per 100 ml. Postmortem examination revealed no anatomic cause for his death.

Comment: It is impossible to be certain that administration of digitalis was responsible for this patient's death. There is, however, circumstantial evidence that would tend to implicate it. First, this man was seventy-five years of age and it is well known that administration of even fractional amounts of digitalis may precipitate serious toxicity in the elderly patient. Debilitation from chronic illness is also a factor enhancing sensitivity to digitalis. The fact that the initial dose of 0.75 mg. digoxin slowed the ventricular rate to 90 per minute and resulted in reversion to normal sinus rhythm is suggestive of increased sensitivity in this patient. While he was losing potassium in the urine

no supplementation of this ion was carried out. In this setting the possibility of an arrhythmia due to digitalis appears plausible. The addition of large doses of digoxin after rate and rhythm had been controlled was based on the widespread practice of administering the average dose for all patients to effect digitalization in a particular patient.

Acid-Base Changes: The characteristic acidbase alterations of the postoperative period have been amply described.1 Most postoperative changes are in the direction of metabolic alkalosis. These prevail if there is no extrarenal loss of base or renal insufficiency. The basis for this alkalotic tendency is the posttraumatic increase in the reabsorption of sodium bicarbonate from the renal tubular fluid. When this alkalosis is associated with hypokalemia (especially in the presence of electrocardiographic evidence of potassium deficiency) there is marked cardiac sensitivity to the toxic action of digitalis. This sequence is most commonly seen in obstructing duodenal ulcer and in pancreatitis.

If respiratory insufficiency, renal insufficiency or extrarenal loss of base (as in small bowel or biliary fistulas) predominate in the clinical picture, then acidosis dominates the postoperative change. There exist no data on the relation of either respiratory or metabolic acidosis on the state of digitalization, whether in the surgical or non-surgical patient.

Changes in Calcium: Earlier, the effects of both increase and decrease in serum calcium concentration on digitalis action were discussed. It was concluded that increasing calcium within the range encountered in patients probably does not alter the action of digitalis on cardiac excitability while decreasing the fraction of ionized calcium tends to protect against digitalis-induced toxic rhythms. There is no evidence that modification of the serum calcium concentration in patients alters the action of digitalis on cardiac contractility. However, experimental evidence would suggest that reduction in serum calcium may compromise cardiac function, and that this can be counteracted by adequate digitalization.54

It is worthwhile to examine briefly the alterations in this cation which may occur in the surgical patient. Changes in serum calcium, phosphorus and phosphatase of a systematic or predictable nature have not been observed after fractures, trauma to soft tissue, anesthesia or operations. Even immobilization, formerly

believed to be a cause of skeletal decalcification, actually results in the loss of only small amounts of calcium daily without any significant change in the serum calcium concentration. There are three factors which may predispose to significant changes in the concentration of this ion: extrarenal loss, parathyroid disease and citrate administration.

Extrarenal loss, as in prolonged intestinal obstruction, can lead to tetany. This is favored by loss of gastric juice and is aggravated by alkalosis. Such a patient may be neither eating nor absorbing calcium. Does such depletion of calcium favor cardiac decompensation? Should such a patient be digitalized? No clear-cut answers are available at the present time although the experimental evidence cited earlier would suggest such a course.

Parathyroid disease as a cause of calcium alteration is, of course, well recognized. Severe hypercalcemia, sometimes seen with metastatic carcinoma of the breast, may also provoke ventricular ectopic beats and enhance cardiac excitability, which may be confused with digitalis toxicity. However, the presence of a shortened Q-T interval and the absence of the S-T segment and T wave changes characteristic of digitalis effect permit electrocardiographic identification of the hypercalcemic etiology of the arrhythmia.

Most important in altering serum calcium level in the operative patient is the use of the citrate ion. Florid citrate intoxication associated with serum citrate levels of 100 to 300 mg. per 100 ml. is rare. In the normal person citrate is metabolized at such a rapid rate that even after massive exchange transfusions no such accumulation occurs. The problems arising from citrate administration are due either to the binding of ionized calcium or to the alkalosis resulting from the production of sodium bicarbonate after the citrate is metabolized.

The standard blood bank procedure employs approximately 30 per cent more citrate than is necessary to bind the calcium in the blood taken from the donor. When multiple transfusions of such blood are given, a reduction in the ionized serum calcium, which cannot be detected by determining total serum calcium, may occur. This reduction in calcium may play a role in the emergence of cardiac decompensation and hypotension in the marginally-compensated patient. It might also reduce the efficacy of digitalis action on cardiac contractility. This important area demands thorough investigation,

but such changes should diminish in significance since they can be prevented completely when calcium is given prophylactically with multiple citrated blood transfusion. As a "rule of thumb," 1 gm. calcium gluconate is given intravenously with each two citrated blood transfusions (1,000 ml.).

SUMMARY

Recognition of the complex interrelations between electrolytes and digitalis action is of vital importance in the management of surgical patients with heart disease. Experimental data bearing on this subject have been examined and the literature reviewed. The following pertinent facts emerge:

- 1. With due recognition that change in a single ion never occurs without other alterations in the total water-electrolyte structure, it is nevertheless clear that in the digitalized patient a decrease in body potassium from whatever cause may precipitate digitalis intoxication. By contrast, administration of potassium will abolish all digitalis-induced arrhythmias even in patients with a presumably normal total of body potassium content. The interaction between digitalis and potassium thus occurs at a cellular level. The cardiac sensitivity to this drug has little relation to the serum potassium concentration.
- 2. However, toxic doses of digitalis interfere with the disposition of potassium within the body. Administration of potassium to patients with advanced heart failure exhibiting digitalis intoxication may cause serious hyperkalemia. The explanation of this fact appears to be twofold: release of potassium by the liver and interference with its uptake by skeletal muscle when under the influence of digitalis. Thus, the treatment of digitalis intoxication with potassium, while essential, carries special hazards in this group.
- 3. Calcium and digitalis have similar actions on the contractility and excitability of the isolated heart. In the intact animal a synergism between calcium and digitalis on cardiac excitability is not demonstrable. Acute reduction in the concentration of ionized calcium results in a hypodynamic myocardium leading rapidly to shock and death. This deleterious action can be prevented either by digitalis administration or by the infusion of calcium salts.
- Magnesium exerts an antiarrhythmic effect on the myocardium and can abolish, transiently, digitalis-induced ectopic rhythms.

When the body is chronically depleted of magnesium by dietary deficiency of this ion, the heart becomes sensitized to the toxic action of

The clinical use of digitalis drugs in the preoperative, intraoperative and postoperative periods is outlined. Simplicity of medication and the avoidance of rapid parenteral digitalization are desirable objectives in surgery. Injudicious rigorous diuresis immediately prior to operation can precipitate serious complications in the digitalized cardiac patient and lead to digitalis toxicity.

The diagnosis and management of cardiac arrhythmias in surgical patients is discussed. Rapid heart action with hypotension in the operative or postoperative period is a particularly difficult problem, and necessitates the differentiation of blood volume deficiency, cardiac arrhythmia, underdigitalization or digi-

talis toxicity as the etiologic factor.

The special problems imposed by posttraumatic metabolism in the cardiac patient are reviewed. The biochemical settings for these problems are: post-traumatic hyponatremia with hyperkalemia, oliguria followed by diuresis, acute acid-base changes, and alterations in ionized calcium.

Finally, it is to be emphasized that the majority of available data dealing with the interrelations between digitalis and body electrolytes concerns only the effects on cardiac excitability. As yet there is a paucity of information concerning the influence of electrolyte alterations on digitalis-induced changes in cardiac contractility. Clearly, much additional investigation is needed on this subject which also bears importantly on the welfare of the surgical patient.

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Historical Milestones

The Treatment of Angina Pectoris by Electricity

(Duchenne, 1855)

Saul Jarcho, M.D. New York, New York

Occasionally it is instructive to examine old therapeutic methods which have become obsolete or unpopular in the course of time and to consider whether such technics have anything to offer to the physician or patient of the present era. These considerations apply to the treatment of angina pectoris by electricity.

Following the discovery of induced electrical currents by Faraday in 1831, Guillaume Benjamin Amand Duchenne de Boulogne (1806–1875) applied them in the treatment of neural and muscular disease. Duchenne thus created the foundations of modern electrotherapeutics. He wrote more than fifty volumes on various aspects of neurology and neurophysiology and made important contributions to the study of locomotor ataxia, the muscular dystrophies and poliomyelitis.

The following excerpt is taken from his famous book "De l'Éléctrisation Localisée et de Son Application à la Physiologie, à la Pathologie et à la Therapeutique," pp. 902–912, Paris, 1855, Baillière.

DUCHENNE ON ANGINA PECTORIS

Since a French physician named Rougnon published, almost a century ago, the first case of angina pectoris,* and the English physician Heberden gave the disease the name which it still bears,† the many authors who have dealt with this disease have concerned themselves predominantly with the nature of the disease and its site.

Thus angina pectoris has been ascribed:

in England to an organic lesion of the heart, especially ossification of the coronary arteries (Jenner, Black, Parry), or a lesion of the aortic arch (Corrigan); in Germany to a rheumatic or gouty cause (Butler, Schoeffer, Hesse, Bergius); in Italy to hypertrophy of the liver causing disturbance or paralysis of cardiac action; and in France (Desportes, Jurine, Lartigue) to neuralgia of the pneumogastric nerve, the cardiac plexus, or the diaphragmatic nerve. The latter authors differ only with respect to the site of the neuralgia. Nowadays each country still defends its own opinions obstinately.

Unhappily it must be admitted that the long and learned discussions which have been conducted concerning the nature and location of angina pectoris have produced no progress whatever in the therapeutics of the disease.

Angina pectoris is the most terrible disease which can threaten human life, for it almost invariably kills after having tortured for a longer or shorter time. How difficult is the position of the physician in the presence of the victim of an anginal seizure, since no therapeutic

* Letter to Lorry on the causes of the death of M. Charles, February 1768.

† Here are the different names which have been given to this disease: angina pectoris, Heberden, 1768; asthma convulsivum, Elsner, 1778; diaphragmatic goutt [sic], Butler, 1791; asthma arthriticum, Schidh, 1793; syncope anginosa, Parry, 1799; asthma dolorificum, Darwin, 1781; sternalgie, Baumes, 1806; sternocardie, Brera, 1810; pneumogastralgie, Téallier, 1826.

‡ Gintrac is the only physician in France who has defended the opinion of the English pathologists. Nevertheless this excellent observer admits that in some cases angina can be essentially nervous (Journal de la

Société de médécine de Bordeaux, 1835).

measure exists which can make the horrible suffering cease immediately, while death threatens to end the attacks which cannot be prevented!

Physicians have not neglected to seek means of fighting the disease. On the contrary, the number of remedies in use is very great and each practitioner acts according to his concept of the nature and location of the disease. The various drugs have sometimes, although rarely, produced an amelioration or even a cure. But from the facts which have been published up to the present time it is apparent that therapeutics is almost powerless against the attack itself and it seems incontestable to me that it is almost always the attack which kills, whether or not the angina is complicated by an organic lesion of the heart or the aorta.

I trust that this brief discussion is enough to show how important it is to stop the anginal attacks, since a single attack can be followed by abrupt death, and to prevent recurrence of the attacks and arrest the advance of the disease.

In the hope of attaining this end I have begun a series of experiments. Here are the first attempts.

CASE 216. Essential angina pectoris of six months' duration. Therapeutic effect of electric stimulation of the nipple and skin: Pérone, aged 50, a currier, lived at no. 25 Rue de Tourtelle, Belleville. He had a strong constitution and a sanguine temperament. He was somewhat plump and short-necked and had never had a severe illness. Two years previously he had had rheumatic pains in the right shoulder which had obliged him to give up work for a month although he had had no fever. He is not usually short of breath, he does not have palpitation; his house is healthful; he is not exposed to dampness.

At nine o'clock on the morning of November 29, 1852, while fasting, he suddenly and for no known reason had a deep burning sensation at the level of the upper and middle parts of the chest and a pain which radiated into the left upper extremity. At the same time he had formications and prickling, which travelled, with increasing intensity, from the elbow to the fingertips. During the attack his heart beat hard and fast. His head felt heavy and hurt a little. It was hard for him to speak since his breath was insufficient and speaking increased the pain. He was forced to bend forward and to stand still or sit. Pain was increased by extending the trunk. Anxiety was extreme. The patient was terrified and felt that his end was near. The first attack did not start to taper off a little until 18 hours after onset, following a large phlebotomy. Before this, mustard footbaths, sedative potions and a full bath had been given, without result. The alleviation however was

not very great, since the patient retained his slight improvement only if he remained seated and absolutely still; the horizontal position would constantly bring back the pain. The most trivial causes would make the pain return; a sneeze, a yawn, or the slightest emotion were enough to cause it. In the daytime he was perfectly at ease except that he had occasional strong attacks lasting eight to ten minutes, produced by movement or emotion. Sleep was impossible. Little by little the attacks became less frequent although as strong as before and as terrifying to the patient and those around him. His appetite and digestion were untroubled and he had no fever during the whole course of the illness. Two weeks after the onset he was given antimonial inunctions over the anterior and upper part of the chest; he was purged every four days; twenty leeches were applied to the anus. Despite this treatment the attacks kept recurring after the slightest exercise, so that the patient was forced to be at complete rest. His physician Dr. Mongeal, seeing that this kept on, decided to refer the patient to me. In the letter which he wrote to me he made the correct diagnosis of angina pectoris and expressed the opinion that contractions of the diaphragm might play a role in the symptoms.

Here are the observations which I made in consultation on April 28, 1853.

In order to come to my office from Belleville, the patient had had to take a wagon. He was unable to climb the two flights of stairs to my office without stopping at each flight and suffering pressure in the chest and the other symptoms already described. After fifteen minutes' rest he felt perfectly well again. Auscultation and percussion revealed nothing wrong in the bronchi, lungs, heart, or great vessels. The pulse was normal. Pressure at any point on the chest caused no pain.

Then I asked the patient to produce an anginal attack. For this it was enough if he bent down as if to pick up something. Here are the phenomena which appeared at the same time. Severe deep burning pain, with a sensation of squeezing, at the level of the upper part of the sternum radiating into the left upper extremity along the posterior part of the arm and the external surface of the forearm into the index finger, with numbness and formication in the entire limb. The patient constantly held his hands crossed against the upper part of the chest, squeezing it, as if to alleviate his suffering. His head was bent forward, his shoulders were held forward and upward by contraction of the pectoralis major and part of the trapezius. When he tried to straighten up or to lower his shoulders, the pain increased. I made him walk but he had not made two steps when the increased sternal pain forced him to stop and sit down. His breathing was short and agitated, his heartbeats violent. His pulse was fast, his face red and engorged. His eyes were wide open. His body was covered with profuse sticky

sweat. His face showed extreme anxiety. Despite this his breath sounds and heart sounds were absolutely normal and percussion of the chest revealed no abnormal dulness.

When the patient attempted to speak his words were choppy and phonation, difficult and weak, increased his pain.

During respiration the movements of the chest and abdomen were perfectly isochronous. There was no pain at the base of the chest and no paralysis of voluntary movement; the left arm and hand were numb and its movements were weak.

After eight or ten minutes' rest everything was normal again but the pain and pressure in the chest were slow to disappear.

The case which I have just described seems to exemplify angina pectoris entirely unassociated with any organic lesion. The burning pain under the sternum accompanied by a sense of compression and squeezing, which precipitated the patient into extreme anguish; this pain which radiated into the left upper extremity following the direction of the radial nerve, with numbness and weakness of the limb and especially of the hand; these attacks provoked by movement or psychologic influences, with no symptoms during the intervals; all these signs warrant my diagnosis. Auscultation and percussion, done with the greatest of care and by several observers, showed clearly that there was no lesion of the heart or of the aortic arch to which the angina might be attributed. Finally the illness presented none of the phenomena observed in asthma, with which angina might be confused in certain cases. Thus the patient did not have the suffocation or the need to draw deep breaths. He merely made his breathing as short as possible, in order to reduce his sternal pain which was aggravated by respiratory movement. There were other symptoms which it would be superfluous to mention, which clearly established the difference between asthma and this patient's angina.

What was the site of the angina pectoris? Was it in the pneumogastric nerve, the cardiac plexus, or the phrenic nerve? I should not like to wander from my subject in order to discuss this question, important though it is. At the same time I must say that, contrary to my statements of 1853 on the possibility of spasm of the diaphragm, the patient presented none of the signs of that condition, hence in the present case the phrenic nerve was not involved in the nervous disorder.

The diagnosis being well established, I come

to the *electrotherapeutic experiments* which were made for the purpose of stopping the anginal attacks and restraining the progress of the disease.

Description of the experiments and results: By making the patient walk I induced a second attack and I applied over the nipple the ends of two metal stimulator wires leading to the conductors of my induction apparatus, which was set at maximum intensity and operated with very rapid interruptions. At the moment when stimulation of the nipple was produced the patient screamed loudly, so that I had to turn off the current. The pain had been very severe but had lasted only an instant. To my great surprise, with the artificial pain which I produced, the pain of the angina also disappeared completely, along with the numbness and formication of the left upper extremity, which ordinarily accompanied it. The breathing quieted down. In short, the patient suddenly found he was well.

Was this sudden change merely the result of coincidence or was it caused by the strong and instantaneous disturbance produced by electric stimulation of the nipple? In order to settle this important question I resumed my experiment and produced a new attack of angina. But this was not as easy as before, since the patient had to make movements of all kinds for four or five minutes in order to make the pain come back, whereas before electric stimulation it was enough if he merely bent down.

The second trial succeeded just as fast as the first, but instead of stimulating the nipple I stimulated the painful area, i.e., at the level of the upper part of the sternum. Since I was happy at being able in this manner to conquer this disease, which had been considered uncontrollable during the attacks, I repeated the trials several times with the same success and I noticed that the more I repeated the stimulation the harder it was for the patient to reproduce his angina, until he finally had to climb rapidly the two flights of stairs in my house in order to bring on an attack.

The next day the patient informed me that he had been able to return home to Belleville without having the least difficulty and without having to stop. He had been able to sleep for the first time since the onset of the disease. In the morning only, he had had pressure without pain, limited to the upper part of the chest. He had come from Belleville on foot and had been able to climb the stairs of my house without stop-

ping and without difficulty. He considered himself cured.

I suggested inducing the angina again in order to apply treatment during the attack as I had done the day before. He made the attempt. It was only after almost a quarter of an hour of great effort similar to those which he made when preparing his hides, that he was able to cause an attack almost as violent as the original seizures. The control of this by electrocutaneous stimulation of the chest was a matter of two or three seconds.

From this day on, the substernal pain and the formication and numbness of the left upper extremity did not return, no matter what was done to reinduce them. The only residue was a sensation of oppression or compression which appeared when he was provoked and which was felt at the former site of pain. Four or five electrocutaneous stimulations at long intervals removed the rest of the angina. Two weeks after the start of the treatment I was able to let the patient return to work.

It is now more than a year since he has returned to his hard work and the angina has never recurred.

It seems to me that two important therapeutic results emerge from the experiments which I have just presented. For one thing, by electric stimulation of sensation of the nipple or skin, applied at the painful area, it is possible to stop an anginal attack completely and instantaneously and to restrain the progress of the disease, perhaps even to cure it definitively.

The first fact is incontestable, for all trials made on this patient during his attacks, whether at their start or during their course, gave absolutely identical results, i.e., they changed the patient's condition suddenly from one of suffering and anguish to one of perfect calm. This achievement is so much the more remarkable because for almost six months the most varied medicines had exerted no effect on the attacks and because, in all the cases reported up to the present time, therapeutics has been almost powerless against the disease.

If the therapeutic method which I bring to the attention of my colleagues were effective only against the attacks, without in the least affecting the progress of the disease, the treatment of angina would still be benefited, since the physician might not only hope to relieve the patients of their horrible sufferings but also perhaps to prevent the terrible death in which the attacks sometimes end. Time would thus

be gained in which to combat the disease by rational methods, which necessarily act slowly or less immediately.

But the therapeutic efficacy of electrical stimulation of the skin and nipple is not limited to this. In the case just cited the attacks rapidly changed their character and intensity under the influence of the repeated trials. Then they occurred less and less often and finally disappeared altogether, despite whatever effort was exerted to make them recur, although for ten months previously the slightest emotion or the least movement were sufficient to bring them on in full intensity.

For more than a year this patient has resumed his heavy work as a currier and has had no more attacks. Can it be said that he is cured? Everyone knows the tendency of angina pectoris to reproduce itself. Hence I must reserve my opinion of this patient, and not pronounce him cured. Time alone can decide. But I feel it has been demonstrated that the treatment which I have tried has powerfully restrained the advance of his angina and I have the basis for hoping that he is cured.

In my opinion the time chosen for electric stimulation of the skin and breast and the repeated reinduction and prompt suppression of the attacks had a favorable influence on the progress of the disease. Here are the ideas on which my procedure is based.

Long experience had taught me that the disturbance caused by electrocutaneous stimulation in a neurosis or neuralgia had the greatest chance of success if it was produced at the very moment of the attack or paroxysm of pain. I had observed, moreover, that the cure of a neuralgia was most solid if the attacks had been most often interfered with in their modality and in their habitual course.

Applying these concepts to angina pectoris, which I consider to be nothing but a neuralgia, I had the idea of provoking in this patient a second attack immediately after having dispelled the first, in order to throw the development of the attacks into disorder. At first I was restrained by the thought that this experiment was not free from danger since no one can tell how an anginal attack might end, but the ease with which I had conquered the previous attack encouraged me to go on. I have already told what happened: it proved very easy to suppress the attacks which I induced, one after another. My predictions were justified by the outcome for the more often the

attacks were broken up during their development, the harder it became for the patient to induce a recurrence.

The truly impressive effect of electrocutaneous stimulation on angina pectoris is explained by the great disturbance of innervation which it causes and by the speed of the shock. Does electricity have an additional effect on the pathological condition of the nervous system? No one would dare to assert this, although it is possible.

If it were only a question of powerful revulsion, fire would be as good as electricity. The latter could not take the place of cutaneous faradization, which does not disorganize the tissues and can be applied safely in any region and prolonged or renewed as often as is necessary.

A second case, for which I am indebted to Dr. Aran, has added to the value of the statements previously presented. I shall give only the main facts:

CASE 217. Mme. X., aged 32, of medium constitution, said that ten years ago, after intense grief following the death of one of her children, she had fallen into a sort of lethargy which lasted a week. While she was in this condition a mirror had to be put before her mouth in order to make certain that she was still breathing. The attack ended in profuse weeping but after this the patient had cardiac palpitations for seven months, with severe anguish, breathlessness, and mental disturbances. Despite the persistence of palpitation the patient's condition had improved, when, two years ago (in 1851), severe grief caused by financial reverses produced a new series of morbid phenomena, which differed from the preceding symptoms in their nature, progress, and intensity. The disease appeared in the form of more or less frequent attacks with apparently healthy intervals. Here are the main symptoms which were observed during each of these attacks: severe precordial pain, which the patient compared to burning intense substernal constriction, with pain which radiated into the left arm and produced numbness there which persisted for some time after the attack and paralyzed the limb completely; extreme anxiety with an expression of terror during the attack. The pectoral muscles and the forward flexors of the neck were contracted. Any attempt to raise the head or move the shoulders backward increased the pain. There was no suffocation as in asthma; respiration was merely short and frequent. The attacks were not accompanied by hysterical phenomena. Thus, there was no constriction of the throat. There were no tears, although it was easy to provoke them by mentioning her child who had died, and then her mind wandered. Auscultation

and percussion revealed no lesion in the lungs, bronchi, heart, or great arterial vessels.

Such was the patient's condition, against which Dr. Aran had fought in vain for a long time when I informed him of the important therapeutic procedure which I have already described. It will be understood that a therapist as distinguished as Dr. Aran would not lose the opportunity of testing the value of a treatment which had succeeded so well in an analogous case, especially since the life of his patient was menaced by increasing danger.

She was subjected to electrocutaneous stimulation during the attacks and obtained as favorable and prompt a result as the previous patient. At present she is almost completely rid of her angina pectoris and has been able to resume her ordinary activities.

Under the influence of intense grief this patient had suffered a series of hysterical attacks during the first and long period of her illness. For a year she had had new and very severe symptoms of a type not observed in hysteria and attributable only to angina pectoris.

This case was less simple than the previous one. It was to be feared that because of the hysterical foundation of the angina, electrocutaneous stimulation might aggravate the symptoms instead of stopping them. Fortunately, these fears proved illusory, since the treatment was almost as successful as in the previous case.

Would the treatment have produced such a result in these two patients if the angina pectoris had been complicated by an organic lesion of the heart or the aortic arch? Experiment alone can decide this question, but in my opinion one can expect if not complete cure, at least some improvement.

I base this statement on the opinion which I have formed concerning angina pectoris from these two cases and from cases in the literature. Everyone admits that angina pectoris can exist as a simple neural disorder. The two cases which I have reported must be added to similar cases already known to science. Very well! When cases of essential angina are compared with those which accompany a lesion of the heart or great vessels, the perfect similarity of the symptoms which appear during the attacks becomes evident. If, on the other hand, the rarity of angina pectoris is contrasted with the frequency of organic lesions of the heart and the aortic arch, we must conclude that angina

pectoris is a condition independent of these organic lesions, although the latter can favor its development or make it more tenacious.

Perhaps it will be objected that I have based the favorable therapeutic influence of electro-cutaneous stimulation in angina pectoris on only two cases. Faithful to the principles which have always guided me in my research, I should doubtless have waited, before publishing, until time and new experiments had given my work more value, were it not that angina pectoris is one of those diseases which can rarely be observed. Moreover, the consideration which has had the greatest influence on my decision in this matter is the powerlessness of therapeutics against the horrible sufferings caused by an anginal attack and the imminence of the danger.

AUTHOR'S COMMENTS

The opening paragraphs of Duchenne's chapter on angina pectoris summarize adequately the diverse opinions which prevailed in his day as to the nature and location of the syndrome. The list of synonyms given in his footnote is additionally illustrative. Duchenne points out that the cleavages of opinion tended to follow national lines.

Duchenne, like many of his colleagues, believed that angina pectoris was a neurosis or neuralgia. These vague terms apparently meant pain originating in the nerves, and not necessarily pain of psychic origin. Duchenne believed that this neuralgia could exist independently of any lesion of the heart and great vessels but that the presence of organic cardiovascular lesions favored the development of angina and made it more difficult to treat.

An outstanding feature of his text is the remarkably detailed description of the anginal attack. The author noted the exact route by which the pain radiated into the patient's arm. He observed the color of the face, the restrained

character of the respiration, the weak voice, the appearance of the sweat and the weakness of the left hand. Such details are not often found in recent cardiologic literature and the physician who is charged with the instruction of interns will struggle in vain to elicit from them the small details, accurately observed and clearly recorded, which contribute to valuable clinical depiction. Aside from the educational value of an insistence on precise observation, the assembly of fine clinical details is important because it provides the investigator with the raw material from which productive research may be initiated.

Although Duchenne repeatedly refers to his therapeutic efforts as experiments, a modern critic would point out the lack of controls. Duchenne considers the possibility that his results may be due to coincidence. He is frank to admit that perhaps he should not have published results based on a series of two cases.

He was of the opinion that electrical stimulation should be given during the anginal attack, since he believed that this disorganized the pattern of pain. He found that the electric current terminated the individual attack instantly and that successive applications made it increasingly difficult to induce new attacks, or as we would say, the threshold of pain had been raised to very high levels. Duchenne wondered whether the effects which he obtained were due to a special action of electricity on the nervous system.

At the present time radical treatment of angina pectoris is mainly in the hands of surgeons and radiophysicists and nothing is said about Duchenne's method. Yet Duchenne's case report suggests that electrotherapy deserves reconsideration either as a therapeutic method or as a device for extending our knowledge of the neural pathways involved in anginal attacks. A modern investigation of the use of electrotherapy in angina pectoris would, of course, require a large series of cases strictly controlled to exclude the factor of suggestion.

Case Reports

Congenital Absence of the Right Pulmonary Artery

Report of a Case in a Five Month Old Infant, with Suggestive Evidence of Unilateral Pulmonary Hypertension*

EMANUEL RUBIN M.D.† and LOTTE STRAUSS, M.D.

New York, New York

YONGENITAL absence of one of the major A branches of the pulmonary trunk has been recognized since 1868 when Frantzel¹ described absence of the right pulmonary artery in association with other cardiac anomalies in a twenty-five year old woman whose death was due to congestive heart failure. During the succeeding eighty-four years only eight additional cases of absence of one of the two pulmonary arteries were reported in world literature, all discovered at autopsy.2-9 Absence of one pulmonary artery was first diagnosed by angiocardiography in 1952.10 Since then the diagnosis has been made repeatedly either by angiocardiography or during surgical exploration or both.11-15 In 1956, Emmanuel and Pattison¹⁶ reported several cases of absence of a pulmonary artery. These and their review of the literature brought the total number of cases reported up to forty-six. Derrick and Howard¹⁷ reported a case emphasizing emphysema of the contralateral lung. Schneiderman¹⁸ added another case with autopsy findings.

To this date only thirteen cases of absence of a pulmonary artery unassociated with other cardiac anomalies have been published; eleven of these presented absence of the right pulmonary artery and two absence of the left pulmonary artery. Of these, four have been verified by autopsy⁴⁻⁶,18 and one by surgical

exploration.¹² In these five cases the right pulmonary artery was absent. The diagnosis cannot be based exclusively on angiocardiography since other conditions such as pulmonary emboli,¹⁹ tuberculosis, cancer,²⁰ atresia and severe stenosis^{20,21} can cause non-filling of a pulmonary artery with radiopaque medium.

The clinical and autopsy findings in an isolated case of congenital absence of the right pulmonary artery will be described, the fifth known instance of this unusual anomaly verified by autopsy. Its interest lies not only in the rarity of the anomaly but also in the fact that it represents an "experiment of nature" offering an unusual opportunity to compare, in one individual, two lungs with different types of blood supply and circulatory dynamics.

CASE REPORT

A three month old white male infant was admitted to The Mount Sinai Hospital for evaluation of congenital heart disease. He was the first child of a twenty-six year old healthy mother, born after an uncomplicated pregnancy of nine months. The birth weight was 3,700 gm. There was no fetal distress during labor, and the baby breathed and cried spontaneously. He was sallow from birth, but never cyanotic. His cry was weak and he did not eat well. Two weeks prior to admission he had a fever of 102° F. and a sore throat. At that time the skin was noted to be slate grey. Fluoroscopy re-

^{*}From the Department of Pathology, Division of Pediatric Pathology, The Mount Sinai Hospital, New York New York.

[†] Fellow of the Dazian Foundation.

vealed an enlarged heart. He was treated with Aureomycin® and penicillin and was discharged after moderate improvement. One week later persistent diarrhea and oral thrush developed and he was readmitted to The Mount Sinai Hospital.

Physical examination revealed a chronically ill infant with ashen grey skin and a weak whining cry. He weighed 4,500 gm. and was 59 cm. long. The circumference of the head measured 39.5 cm.; the chest measured 35.5 cm. The temperature was 102°F., the heart rate 132 per minute and the respiratory rate 40 per minute. He had no edema and no definite cyanosis. The fontanelles were not bulging. The pupils were regular, equal and reacted to light. A shaggy white adherent material was seen on the buccal mucosa, tongue and posterior pharyngeal wall. The lungs were clear to percussion and auscultation. The left border of cardiac dullness was almost at the anterior axillary line. He had a regular sinus rhythm and the heart sounds were of good quality. P₂ was greater than A₂. A grade 3, harsh, systolic murmur was heard best in the third and fourth intercostal spaces along the right sternal border. The edge of the liver was felt 3 cm. below the right costal margin. The tip of the spleen was palpable. The extremities were flaccid and the reflexes weak.

Laboratory Findings: Hemoglobin was 16 gm./100 ml., leukocyte count 17,600/cu. mm., with 54 per cent polymorphonuclear cells and 45 per cent lymphocytes. The urine was non-contributory. Stool culture yielded Escherichia coli and Pseudomonas pyocyanea. Pharyngeal culture yielded P. pyocyanea and Aerobacter aerogenes. A blood culture was negative. Candida albicans was recovered from the buccal mucosa. Radiographic examination of the chest revealed an increase in the transverse diameter of the heart and of the convexity of the left heart border, with elevation of the apex. The hilar and peripheral pulmonary vessels appeared within normal limits, and the superior mediastinum was somewhat prominent. An electrocardiogram revealed sinus tachycardia and right axis deviation, deeply inverted T2 and T3, a prominent R wave in aVR, and a deeply inverted T wave in aVF, RR' pattern and inverted T in leads V1 to V3, and a deep S in leads V₃ to V₆. These findings were interpreted as suggesting enlargement of the right ventricle with myocardial involvement.

Clinical Course: The diarrhea did not respond to intravenous therapy but gradually subsided when the diet was changed to a meat-based formula. The patient received digitalis, but increasing congestive heart failure developed. In the fifth week of hospitalization, he began to have periods of apnea, cyanosis and bradycardia. Rales developed at the left apex and his liver enlarged. His condition gradually deteriorated, and at the age of five months he suddenly died following an attack of apnea and bradycardia.



Fig. 1. Abdominal and thoracic situs showing enlarged liver and prominence of the right ventricle.

AUTOPSY FINDINGS

The body was that of an undernourished boy weighing 4,100 gm., with slight cyanosis of the lips and nailbeds. With the exception of an enlarged liver (Fig. 1), the pertinent findings were restricted to the thoracic organs. The heart weighed 57.5 gm. (normal weight, 29 gm.). It was enlarged with prominence of the right ventricle (Fig. 1). The foramen ovale was patent, although well curtained by a loose thick valve which bulged into the left atrium. The right ventricle was dilated and hypertrophied; the infundibulum was wide, and the pulmonary valve ring, which was guarded by three wellformed semilunar values, measured 3.3 cm. in circumference (Fig. 2). The wall of the right ventricle was 1 cm. thick. The pulmonary veins entered the left atrium which was not enlarged. The left ventricle was within normal limits of size. The aortic valve ring measured 2.8 cm. in circumference.

The pulmonary trunk was dilated, its/diameter exceeding that of the aorta. The intima was smooth. The main pulmonary artery failed to divide into right and left branches, but turned to the left lung; its major branches also were dilated. No rudiment of a right pulmonary artery and no ligamentum arteriosum was found. The aorta was of normal caliber and had a smooth intima. About 5 mm. proximal



Fig. 2. Heart after opening: to the left is seen the hypertrophied right ventricle with dilatation of the outflow tract and the pulmonary trunk.

to the origin of the innominate artery, a funnel-shaped opening in the lateral wall of the arch of the aorta narrowed into a blind cord which could be traced to the right, downward to the hilus of the right lung, anterior to the main bronchus. The atretic portion of the vessel was 1.5 cm. long. It became patent distally, and divided into branches to the three lobes of the right lung at the hilus. These arteries were much smaller and thinner than the

A YEUR B

Fig. 3. Schematic drawing illustrating the anomaly of the pulmonary arteries. A single large pulmonary artery supplies the left lung. A, atretic systemic artery to the right lung arising from arch of aorta. B, dilated bronchial artery supplying right lung. Note difference in size between the two lungs.

arteries of the contralateral lung which was supplied by the pulmonary artery. The main branches of the aortic arch were normal. One of the three bronchial arteries was dilated and could easily be traced to the hilus of the right lung behind the right main bronchus. At its origin from the aorta the lumen measured 1 to 2 mm. The ostia of the right intercostal arteries were about twice the size of those of the left. The anatomy of the heart and large vessels is schematically illustrated in Figure 3.

The combined weight of the two lungs was 140 gm. (normal weight: 75 gm.). The right lung was about one-half the size of the left lung. The right lung possessed three lobes, the left lung two. Firm, fibrous adhesions obliterated the right pleural space. The right lung was pink and appeared fairly well aerated. The left lung was red, poorly aerated, and had a meaty consistency. Scattered throughout the upper lobe of the left lung were numerous, discrete, pale, raised areas.

Microscopic Description: The two lungs differed considerably in their microscopic structure. The right lung showed uneven aeration with patchy atelectasis, especially in the subpleural zone. The pleura was thickened with proliferation of fibrous tissue and numerous small arteries. The lung contained multiple nodular foci of chronic interstitial pneumonitis with local fibrous thickening of the alveolar septums. Elsewhere the alveolar septums were thin, and the capillaries were not engorged. Some alveoli contained collections of mononuclear inflammatory cells. A few small foci of hemorrhage were noted. The blood vessels were thin-walled throughout, and the small arteries had a wide lumen (Fig. 4A).

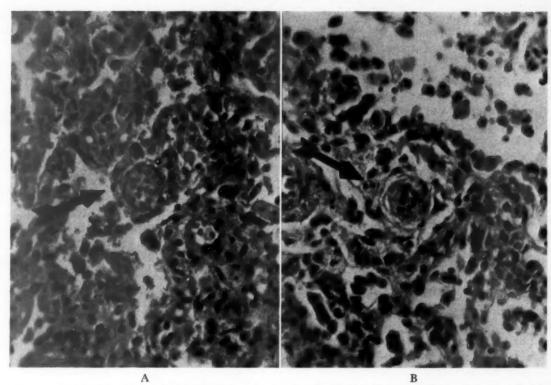


Fig. 4. A, photomicrograph of small artery of right lung. The vessel is thin-walled and has a wide lumen. B, photomicrograph of small artery of left lung. Note medial thickening and narrow lumen. Hematoxylin and eosin, \times 430, enlarged from 35 mm.

The left lung revealed poor aeration and diffuse engorgement of the capillaries. Numerous areas of recent hemorrhage were present, and many of the air spaces were filled with hemosiderin-laden macrophages. This lung showed diffuse thickening of the alveolar septums, with early fibrosis manifested by increase in the number of reticular fibers. The elastic arteries were dilated and their structure appeared normal. The small muscular arteries showed hypertrophy of the media and a narrow lumen (Fig. 4B) in striking contrast to comparable vessels of the opposite lung. Intimal proliferation was not seen.

Cross section through the atretic portion of the anomalous systemic artery to the right lung from the arch of the aorta showed intimal proliferation and occlusion by old calcific thrombotic material containing hemosiderin pigment. The myocardium of the right ventricle was hypertrophied.

COMMENTS

The anomaly presented in this case strikingly resembles previous descriptions of isolated absence of the right pulmonary artery. All the autopsy reports concern infants under one year, while those cases in which the diagnosis has been based only on angiocardiographic examination represent older children or adults. This would indicate that the anomaly is not

necessarily fatal in early life, provided that angiocardiographic diagnosis can be accepted without anatomic confirmation. During life, the diagnosis of congenital absence of one pulmonary artery is made best by angiocardiography, where non-filling of one of the main branches of the pulmonary trunk is observed. Demonstration of an anomalous artery to the affected lung is more conclusive.

Demonstration of Anomalous Systemic Artery: In all but one of the isolated autopsy cases of absence of the right pulmonary artery, an anomalous artery has been demonstrated which arises from the aorta proximal to the origin of the innominate artery and leads to the hilus of the right lung. In one case report⁵ no mention is made of the arterial blood supply to the right lung. Surgical exploration in one instance demonstrated the anomalous systemic artery to the right lung which measured 4 mm. in diameter.12 In most of the cases in which the anomaly was diagnosed by angiocardiography, definite filling of an anomalous systemic artery could not be demonstrated. The discrepancy between the anatomic and the radiologic findings suggests caution in basing the diagnosis

on radiologic evidence alone. In the case presented here an anomalous systemic artery to the right lung was present at the usual site, which, however, was occluded at the time of the child's death. The main blood supply to the right lung was furnished by a dilated bronchial artery. In addition, numerous newly formed collateral channels were noted, probably derived from the right dilated intercostal arteries.

The lung which derived its blood supply from the systemic circulation was considerably smaller than that supplied by the pulmonary artery. The small size of the lung, which has no pulmonary artery, has been attributed to shrinkage caused by fibrosis, 23 and the large size of the normally supplied lung to emphysema. 17 In the absence of fibrosis of the right lung or of emphysema in the left lung, in the case under discussion, it is believed that the right lung was congenitally small, probably as a result of diminished vascular supply.

Difference in Vasculature of the Two Lungs: The difference between the structure of the smaller pulmonary vessels in the two lungs deserves comment. The small muscular arteries and arterioles of the left lung had a thickened media and decreased lumen-to-wall ratio, resembling fetal pulmonary vessels. In the right lung the corresponding vessels had a thin media and a greater lumen-to-wall ratio. High pulmonary arterial resistance is physiologic in fetal life, but after birth the structure of these vessels gradually changes from the thickwalled "fetal" type to the thin-walled mature type. This development is usually complete by three months.22 However, when the pulmonary arterial pressure remains high, as in some forms of congenital heart disease where the pulmonary vascular bed is subjected to systemic blood pressure, the thick-walled fetal structure of the vessels may persist.23 In the case under discussion, the presence of arteries of the fetal type in the left lung alone together with right ventricular hypertrophy suggest hypertension in the left lung which received the entire cardiac output of the right side of the heart. On the other hand, the right lung, which received a much smaller portion of the cardiac output, revealed thin-walled muscular arteries with a wide lumen. While right ventricular hypertrophy has been described in other reports of isolated congenital absence of one pulmonary artery, the vasculature of the two lungs has not been described in previous

reports of this anomaly. As far as the right lung is concerned the state of the small arteries probably depends to some extent on the size of the systemic vessel supplying the lung, i.e., whether the systemic blood pressure can make itself felt in the abnormally supplied lung.

Causes of Unilateral Pulmonary Hypertension and Right Ventricular Failure: Virchow, in 1848,24 first showed that one branch of the main pulmonary trunk may be ligated without necrosis of the lung which it supplies if the bronchial circulation is intact. Subjects with absence of one pulmonary artery generally have normal oxygen saturation of the blood10,25 and normal pulmonary artery pressure.11,26 Why then do some patients with this anomaly die in early infancy with signs of right ventricular failure and anatomic changes suggestive of hypertension in the "normal" lung? In the patient described herein these consist of right ventricular hypertrophy and the persistence of the "fetal" structure of the small pulmonary arteries in the left lung. Thickening of the alveolar septums caused by chronic congestion and repeated pulmonary infections may have been contributing factors in the causation of increased resistance in the pulmonary circulation. Furthermore, one should consider pulmonary vasoconstriction which has been demonstrated in animals.27,28 DeBurgh Daly²⁹ demonstrated vasoconstrictor and vasodilator fibers, whose stimulation results in a change in the caliber of pulmonary vessels. Animal studies have established that the tone of the pulmonary vessels can be influenced by pharmacologic agents, the most potent of which is serotonin. 30 Suggestive evidence for pulmonary vasoconstriction in man has been presented by Gorlin⁸¹ and Patel.32 It may be that in susceptible infants the pulmonary arteries, the expansibility of which is already limited by a distended lung, react to the abnormally increased blood flow with vasoconstriction.83,34

The cause of the persistence of "fetal" type vessels is not known, but their association with pulmonary hypertension is well documented.²³ An alternate explanation for the presence of thick-walled vessels in cases of pulmonary hypertension has been advanced by Evans and Short,³⁴ who maintain that these vessels are not persistent fetal arteries but represent a diffuse arterial contracture as a result of persistent and diffuse organic constriction. In any event one might postulate that increased flow in the left lung of this child initially led to

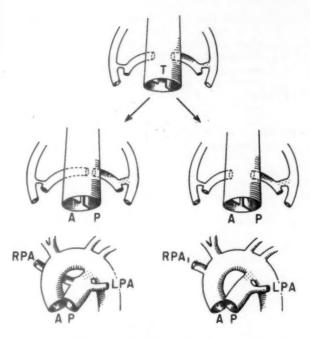


Fig. 5. Diagram showing relation of sixth aortic arch to truncus arteriosus and development of pulmonary arteries; left side normal, right side abnormal. (Adapted from: Schneiderman, L. J. Am. Heart J., 55:772, 1958. 18)

increased tone in the pulmonary vascular bed, i.e., to vasoconstriction. This would tend to maintain a high pulmonary pressure and eventually lead to permanent structural modifications in the small pulmonary arteries and arterioles, thus producing a further elevation of pressure in the lesser circulation. In the right lung, which was supplied by systemic blood vessels, the blood flow must have been far less than in the left lung, as evidenced by the absence of congestion in the former. The structure of its blood vessels did not differ from that seen in lungs under normal conditions of circulation. The inherent responsiveness of the pulmonary vessels to altered circulatory dynamics probably varies, and the patients with this anomaly who survive beyond infancy may be those who do not respond to the increased pulmonary blood flow with vasoconstriction, and consequently do not have pulmonary hypertension. On the other hand, it may be said that vasoconstriction may act as a protective mechanism against flooding of the pulmonary vascular bed in the presence of increased blood flow. Without actual measurements of blood flow and pressures, the deductions made from the observation of the structural differences

in the vessels in the right and left lung remain somewhat speculative.

In analogy to the situation in this congenital anomaly one may recall a case reported by Evans²⁵ in 1913, of a thirty-nine year old woman with massive pleural adhesions and collapse of the right lung and emphysema of the left lung. This patient had pronounced right ventricular hypertrophy with unilateral pulmonary atherosclerosis and hypertrophy of the muscular arteries of the emphysematous left lung, while the arteries of the collapsed right lung were normal. This represents an instance of acquired unilateral pulmonary hypertension.

Embryology: The embryogenesis of the pulmonary arteries has recently been reviewed by Schneiderman¹⁸ who offers a plausible theory for the abnormal development resulting in congenital absence of the right pulmonary artery. This is schematically shown in Figure The pulmonary arteries develop from the sixth aortic arch.36,37 In normal development the right sixth arch shifts so far to the left that, as the truncus arteriosus divides, both pulmonary arteries come to originate from the pulmonary side of the truncus. If this shift to the left of the right sixth aortic arch has not taken place by the time septation of the truncus is complete, only the left pulmonary artery arises from the pulmonary trunk, while the vessel which should have become the right pulmonary artery is caught on the aortic side of the truncus. This theory not only would account for the absence of a normal right pulmonary artery, but also for the origin of the anomalous artery to the right lung from the arch of the aorta.

SUMMARY

The clinical history and anatomic findings in a case of isolated congenital absence of the right pulmonary artery are described and the literature reviewed.

Medial hypertrophy and narrowing of the lumen of small arteries only in the lung supplied by the pulmonary artery, together with hypertrophy of the right ventricle, are interpreted as evidence of unilateral pulmonary hypertension. It is suggested that hypertension in the left lung may be the result of vasoconstriction in response to increased blood flow.

ACKNOWLEDGMENT

We wish to thank Dr. Sidney Blumenthal for permission to report this case.

ADDENDUM

Since submission of the manuscript our attention has been called to another report of absence of the right pulmonary artery complicated by a patent ductus arteriosus, diagnosed during life. Biopsy of the two lungs showed vascular changes in the lung supplied by the systemic artery. 8

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Primary Thrombocytosis and Anginal Syndrome*

ARTHUR BERNSTEIN, M.D., F.A.C.C., FRANKLIN SIMON, M.D., EDWIN L. ROTHFELD, M.D.†

and Frederick B. Cohen, M.D.

Newark, New Jersey

NGINA pectoris is frequently ascribed solely to coronary atherosclerosis without further consideration of any other pathogenesis. The fact that other entities, such as thyrotoxicosis, thromboangiitis obliterans and aortic valvular disease, may be etiologically related is often overlooked. We have recently observed two relatively young patients in whom a blood dyscrasia, primary thrombocytosis, was a probable etiologic factor. Following therapy with radioactive phosphorus, anginal complaints and platelet counts decreased in a parallel manner associated with improvement in the electrocardiogram. After varying intervals, angina, thrombocytosis and electrocardiographic changes of an ischemic nature recurred. Although angina pectoris with polycythemia has been described, this symptom has not been previously mentioned with myeloproliferative disease involving only platelets.

CASE REPORTS

Case 1. A forty year old white man was admitted to the Newark Beth Israel Hospital on January 24, 1957. The chief complaint was pain in the chest. This pain was substernal in location and "mild" in intensity at the time of its onset, ten days prior to admission. Eight days later, it became severe and crushing in nature, radiating to the left arm and persisting about an hour. Subsequent to this episode, the patient noted intermittent pain in the chest of a less severe degree. The physical examination at the time of admission was non-contributory. The blood pressure was 140/80 mm. Hg, pulse, 84 per minute.

On January 26, 1957, the following data were obtained: 8,600 white blood cells per cu. mm.; 5.51 million red blood cells per cu. mm.; 92 per cent hemoglobin (Sahli); 51 per cent hematocrit and 632,000 platelets per cu. mm. Radioactive blood volume determination on January 28, 1957, was 68.5 cc. per kg. Urinalyses, routine blood chemical studies and

roentgenograms of the chest and gastrointestinal tract were within normal limits.

The pain in the chest persisted throughout the patient's hospital stay, although it was not severe. Serial electrocardiograms revealed T wave inversion in the left precordial leads and extremity leads II, III and aVF compatible with myocardial damage secondary to coronary insufficiency (Fig. 1).

Clinical Course: Because of the moderate thrombocytosis, a dose of 4 mc. of P32 was administered orally on January 29, 1957. The patient was discharged one day later. He became free from pain in about six weeks and returned to work as a rug-cleaner on March 15, 1957. During this interval, the platelet count diminished to normal values, and the T wave changes in the electrocardiogram reverted almost to normal. On May 13, 1957, he again noted substernal distress on walking two to three blocks. The platelet count had risen to 466,000 per cu. mm. but the electrocardiogram showed only minor changes. On May 24, 1957, another dose of P32 was administered orally. Six weeks later he again was free from angina and was even able to play tennis. On August 5, 1957, the platelet count was 264,000 per cu. mm., the electrocardiogram showed only minimal T wave flattening in leads V5 and V6, and the patient had no complaints. He was not seen again until December 9, 1957, when he complained of persistent severe angina associated with a platelet count of 786,000 per cu. mm. The electrocardiogram at this time was unchanged. Another dose of P32 was given on January 7, 1958.

Comment: This case may be summarized by stating that there was no obvious cause for the angina either in the patient's family history or in the laboratory investigation. The platelet count was made in a search for factors which might have precipitated angina in an otherwise healthy man with apparently minimal atherosclerosis. The elevation of the platelet level with no other hematologic disturbance was an unexpected finding which was not accepted as

^{*} From the Departments of Medicine and Hematology, Newark Beth Israel Hospital, Newark, New Jersey.
† Public Health Service Research Fellow of the National Heart Institute, Newark Beth Israel Hospital.

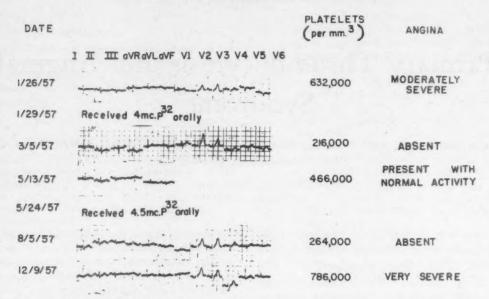


Fig. 1. Electrocardiograms of Case 1. Note improvement in T wave abnormalities following initial P⁸² therapy. Subsequent electrocardiograms show little correlation with either platelet levels or anginal complaints.

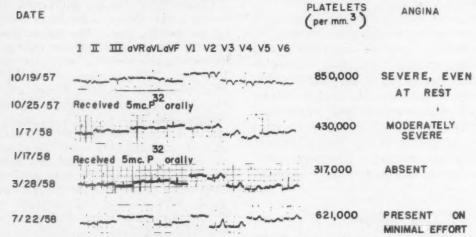


Fig. 2. Electrocardiograms of Case 2. As in Case 1, T wave abnormalities revert to normal following initial P⁸² therapy, but subsequent electrocardiograms fail to show correlation with the level of the platelet count or severity of the angina.

a precipitating factor until the parallelism between symptoms and platelet levels became apparent (Fig. 1).

CASE 2. A forty-five year old white man was admitted to the Newark Beth Israel Hospital on October 18, 1957. He complained chiefly of pain in the chest of three weeks' duration. He had been told that his blood pressure had been elevated for the past two years. About three weeks prior to admission he noted the spontaneous, progressive onset of a dull pain in the left side of the chest which radiated to the shoulder and down the left arm. This pain was aggravated by exercise, relieved by rest and associated with mild dyspnea and generalized weakness.

Physical examination revealed an obese man in no distress. The radial pulse was 80 per minute; the

blood pressure in the left arm, 158/95 and in the right arm, 150/95 mm. Hg. Except for an occasional extrasystole and evidence of exogenous obesity, the remainder of the examination was non-contributory.

The following data were obtained on October 19, 1957: 7,000 white blood cells per cu. mm.; 5.99 million red blood cells per cu. mm.; 110 per cent hemoglobin (Sahli); 850,000 platelets per cu. mm. Radioactive blood volume determination on October 19 was 48.6 cc. per kg. Further studies, including roentgenogram of the chest, urinalyses, routine blood chemistries and intravenous pyelogram, were normal.

Substernal pressure on effort persisted through most of the hospital stay, unchanged in intensity. Serial electrocardiograms showed inverted and progressively deepening T waves compatible with myocardial damage (Fig. 2).

Clinical Course: On October 25, 1957, 5 mc. of P32 was administered orally. His anginal complaints and platelet count decreased, and the electrocardiogram showed a striking improvement. On January 17, 1958, he was again given 5 mc. of P32. He became asymptomatic for the first time on March 28, 1958, when the platelet count fell to 317,000 per cu. mm. Angina on minimal effort reappeared about four months later when the platelet count had again risen to 621,000 per cu. mm. However, the electrocardiogram at this time showed no change. Another dose of P32 on September 5, 1958 was effective in lowering the platelet count and diminishing the complaints. On December 23, 1958, the platelet count was 291,000 per cu. mm. and the patient was free of symptoms.

Comment: In this case, too, symptoms and platelet levels ran parallel courses although the electrocardiograms did not always show changes when symptoms were prominent and platelet counts were high (Fig. 2).

COMMENTS

Nygaard and Brown,1 in 1937, were the first to describe essential thrombophilia. They reported five cases in which multiple thromboses occurred, involving the extremities, heart, lungs, kidneys, pancreas, eyes and central nervous system. There was no evidence of vascular disease and platelet counts were normal or only moderately elevated, except one case in which platelets numbered over 2 million per cu. mm. They were of the opinion that thrombosis was due to loss of "suspension stability" of platelets related to an increased globulin-fibrinogen fraction in the plasma proteins. Epstein and Richter2 described another case in which thrombosis involved the extremities, spleen and pancreas. The globulin fraction of the plasma proteins was increased, but unfortunately no platelet counts or coagulation studies were performed. In the case reported by Monto et al:,3 thromboses involved the extremities and lungs in the presence of normal vascular structures. Studies revealed a moderate thrombocytosis, increased blood coagulability in vitro, increase in the globulin and fibringen fractions of the plasma proteins and megakaryocytic hyperplasia in the bone marrow.

Essential thrombocythemia or thrombocythemia hemorrhagica is characterized by markedly elevated platelet counts, usually in the millions. There is a tendency toward venous thrombosis and spontaneous hemorrhage.⁴ The bleeding time is prolonged; there is mega-

karyocytic hyperplasia in the marrow, and occasionally moderate leukocytosis and splenomegaly. Fanger et al.⁵ reviewed the reports of twenty-eight such cases and concluded that thrombocythemia is a myeloproliferative disorder intimately associated with other entities such as leukemia and polycythemia vera. In the case reported by Smith⁶ there was profuse hematuria and hematemesis with high platelet and megakaryocyte counts and evidence of extramedullary hematopoiesis, as proved by liver biopsy.

It seems safe to assume that these syndromes (thrombocytosis, thrombocythemia, essential thrombophilia, etc.) represent variants of a single myeloproliferative disorder limited primarily to the platelet series. The nomenclature is, therefore, relatively unimportant. The clinical manifestations are related to thromboses in large and small vessels and depend on the structures involved.

Our case reports depict two patients in whom the clinical course and electrocardiographic abnormalities of coronary artery disease seemed to vary directly with the degree of thrombocytosis. A diagnosis of polycythemia was not considered because no significant erythrocytosis, leukocytosis or hypervolemia could be demonstrated in either case. There was no apparent cause, such as infection or trauma, for the elevated platelet counts and, therefore, primary thrombocytosis seemed to be the most reasonable title for this disorder. Of major interest is the fact that only the coronary vessels were apparently involved by a generalized hematologic disorder which presented no signs or symptoms suggestive of reticuloendothelial dis-The most likely explanation for the symptomatology is that pre-existing coronary atherosclerosis formed a favorable nidus for loose platelet agglutination or adhesion, producing narrowing of the coronary vessels and insufficiency. In these instances, therefore, thrombocytosis was probably a complicating rather than an initiating factor. Platelet thrombi or masses probably involved only the smaller divisions of the coronary vessels so that the clinical and electrocardiographic abnormalities were those of angina and coronary insufficiency rather than of extensive myocardial infarction. There was reversal of the picture when the platelet level was reduced.

The variability in response to therapy with radioactive phosphorus deserves mention. In Case 1, pain in the chest was absent about six weeks after the initial dose, and the platelet count was approximately one-third the pretherapy level at that time. Substernal pain was noted again nine weeks later, associated with an elevated platelet count, 466,000 per cu. mm. (normal: 200,000 to 300,000). A second dose was administered, and the patient then remained free of angina for six months.

In Case 2, the initial dose caused only a moderate decrease in platelet levels and anginal complaints, although the electrocardiogram showed striking improvement. A second dose of radioactive phosphorus administered three months later caused a decrease in pain in the chest within ten weeks, when the platelet count had fallen to 317,000 per cu. mm. Six months after the second course of therapy, angina and thrombocytosis were again evident.

There was no significant effect of this therapy on erythrocyte or leukocyte levels in either case. No untoward side effects were observed. Since radioactive phosphorus is usually effective in reducing elevated counts, and in both of these cases was able to reverse the symptomatology, the diagnosis of this disorder is of more than academic interest. It is, therefore, suggested that hematologic studies, including platelet counts, be performed in patients with clinical evidence of coronary artery disease; particu-

larly when the youth of the patient and the absence of diabetes, hypercholesterolemia and hypertension make the possibility of advanced atherosclerotic changes unlikely.

SUMMARY

Two cases of angina pectoris complicated by primary thrombocytosis in which reduction of the platelet count with radioactive phosphorus relieved the angina are reported.

Hematologic studies including platelet counts should be performed on young patients with symptoms and signs of coronary artery disease.

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Hemopericardium Following Rupture of a Bacterial Aortic Sinus Aneurysm*

J. D. Bristow, M.D., Brent M. Parker, M.D.† and Walter A. Haug, M.D.‡
Portland, Oregon

It is the purpose of this paper to report an unusual case in which bacterial endocarditis led to the development of an aneurysm of the left aortic sinus of Valsalva with fatal rupture into the pericardium.

CASE REPORT

History: A thirty-three year old white man entered the Portland, Oregon, Veterans Administration Hospital on November 25, 1957. He was confused and the patient's history was therefore obtained from his wife and his private physician. When the patient was twenty-one years old he had what appeared to be his first attack of acute rheumatic fever. In the five years prior to this hospitalization he had often complained of aching joints, but a recurrence of rheumatic fever had not been diagnosed. Three months before admission the patient again suffered from painful joints. He was seen by a physician and hospitalized for active rheumatic fever. Treatment included administration of adrenal corticoids. Two weeks later the patient returned to work and it is not known whether administration of corticoids was continued.

Two weeks before admission to our hospital he complained of pain in the chest and had a temperature of 106° F. He entered a hospital in another city where a diagnosis of rheumatic heart disease with bacterial endocarditis was established. Pericardiocentesis was performed and yielded 300 cc. of cloudy yellow fluid; Staphylococcus albus was isolated from this fluid and the blood. The organism was sensitive to most antibiotics. Despite a history of allergy to penicillin, penicillin was administered orally since this agent was considered to be the drug of choice. Two days after this therapy was begun a sudden episode of diaphoresis, dyspnea and shock occurred which was terminated by the intravenous administration of hydrocortisone. Treatment with the antibiotic agents novobiocin, tetracycline and chloramphenicol was then started. A second pericardiocentesis produced a scant amount of bloody fluid.

The patient continued to have a febrile course and during the last week had received erythromycin in addition to his previous drug therapy. He was also given a daily maintenance dose of digitalis. One day prior to transfer to our hospital he became disoriented and his temperature rose to 104°F.

Physical Examination: On admission the patient appeared to be critically ill and complained of pain in the entire anterior part of the chest. He was diaphoretic, had mild cyanosis and was lethargic. The pulse rate was 120 beats per minute, the blood pressure 112/58 mm. Hg and the oral temperature 100.2°F. In the left malar area there was a reddened, firm skin lesion, 1 cm. in diameter. A petechial hemorrhage was present in the left buccal mucosa. No venous distention was found. Flatness to percussion was present in the left lower half of the chest posteriorly joining with the area of cardiac dullness in the left axilla.

The cardiac apical impulse was visible and palpable in the sixth intercostal space in the anterior axillary line and was of strong quality. Systolic thrills were present in the aortic and apical areas. A grade 3 rough systolic murmur was heard in the aortic area with transmission into the neck and along the left sternal border. In the third left intercostal space adjacent to the sternum a grade 1 early diastolic decrescendo murmur was audible. A pericardial friction rub was present at the lower left sternal border. The edge of the liver was percussed four cm. below the right costal margin but neither the liver nor the spleen was palpable. There was no edema and all peripheral pulses were present. No localizing neurologic signs were found. Slight lymphadenopathy was noted in the posterior cervical and axillary areas. The venous pressure in the antecubital fossa was 12 cm. of saline, with a rise to 16 cm. following hepatic compression.

Laboratory Findings: The hemoglobin was 10 gm. per cent; sedimentation rate was 39 mm. in one hour; white blood count was 29,000 per cu. mm. with 88 per cent neutrophils; blood urea nitrogen was 28 mg. per cent. Urinalysis disclosed 3 plus albumin and a few

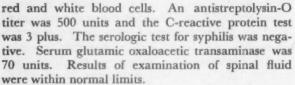
From the Departments of Medicine and Pathology, Veterans Administration Hospital, Portland, Oregon.

[†] Present address: Washington University School of Medicine, St. Louis, Missouri.

Present address: St. Anthony's Hospital, Pendleton, Oregon.



Fig. 1. The heart and pericardium. The pericardial sac with its massive blood clot has been reflected to the right of the illustration. The heart, which has been longitudinally incised, is on the left. The entire specimen shown here weighed 2,350 gm.



Marked cardiomegaly, with the heart filling the entire lower left side of the chest, was revealed by an anteroposterior roentgenogram of the chest. An electrocardiogram showed slight S-T segment elevation and T wave inversion in lead aVF. The precordial leads displayed moderate S-T segment depression and upright T waves from leads V₂ to V₆. First degree A-V block was present. In view of later findings it is interesting that left ventricular hypertrophy was not evident on the electrocardiogram.

Course: The patient was critically ill at the time of admission. The diagnoses of rheumatic heart disease with aortic stenosis and insufficiency and bacterial endocarditis seemed apparent; a complicating bacterial pericarditis was also considered. Initially, oxygen and analgesic agents were administered. On pericardiocentesis, performed three hours after hospitalization, only 30 cc. of sanguinous fluid could be obtained, the hemoglobin content of which was 3 gm. per cent. Antibiotic therapy with erythromycin and chloramphenicol was begun. Five hours after admission clonic movements of the upper extremities suddenly developed and the patient died within a few minutes. Six blood cultures, a pericardial fluid culture and a spinal fluid culture subsequently failed to show bacterial growth.

POSTMORTEM FINDINGS

The external appearance was that of a well developed white man. The major pathologic findings



Fig. 2. Close-up view of the aortic valve. The valve has been partially incised. Nodularity and thickening of the cusps are seen as well as fusion of the commissures. Vegetations are present. The arrow indicates the opening into the aneurysm of the left aortic sinus which communicated with the pericardial space.

were limited to the thoracic and abdominal regions.

The left pleural space contained 500 cc. of clear, brownish colored fluid, and the right held 50 cc. The unopened pericardial sac was greatly distended and its contents weighed 2,350 gm. When the sac was incised, the pericardial cavity was found to be filled with old and new clotted blood, which accounted for the great size (Fig. 1). It was apparent that not all of the intrapericardial bleeding was acute. A diffuse fibrinous pericarditis covered the heart.

After removal from the pericardium, the heart weighed 750 gm. The right ventricular wall was 5 mm. in thickness and the left wall was 20 mm. thick. No evidence of myocardial infarction was found. On palpation, there was minimal thickening of the commissures of the mitral valve. The tricuspid and pulmonic valves were normal.

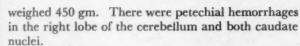
There was fusion of all commissures of the aortic valve with marked scarring, nodularity and rigidity of the cusps. Calcification of the valve was evident. The left coronary cusp was covered with brown friable material. There was an opening 1.5 cm. in diameter in the aortic wall posterior to the left coronary cusp, leading into an aneurysm of this aortic sinus (Fig. 2). The aneurysm communicated with the pericardial cavity, with an opening between the aorta and superior vena cava, and anterior to the right pulmonary artery. Thus, it was concluded that a ruptured aneurysm of this aortic sinus was the cause of the patient's hemopericardium.

A 2 by 1 cm. ulceration, covered with granulation tissue, was present on the septal wall of the left atrium, but no perforation had occurred there.

The liver weighed 3,400 gm. and cut sections of it displayed a "nutmeg" appearance. The spleen



Fig. 3. Photomicrograph of a section from the aortic valve. Acute endocarditis is present as evidenced by the inflammatory exudate and large dark-staining bacterial masses. Valvular scarring and calcification are seen in the lower part of the photograph.



Microscopic sections of the aortic valve demonstrated acute inflammatory exudate, granulation tissue, fibrosis and calcification (Fig. 3). Numerous large colonies of bacteria were present, which on smear were gram-positive cocci. The sections of myocardium displayed scattered areas of acute inflammation with segmented neutrophils in some regions forming microabscesses (Fig. 4).

Culture of material removed from the aortic valve grew coagulase positive Staphylococcus aureus.

The final pathologic diagnoses were (1) rheumatic heart disease with aortic stenosis; (2) S. aureus endocarditis of the aortic valve and left surface of the atrial septum; (3) mycotic aneurysm of left coronary sinus of Valsalva with rupture into the pericardium; and (4) bacterial myocarditis.

COMMENTS

Aneurysms of the aortic sinuses are most commonly of congenital origin^{1,2} and are frequently associated with other developmental anomalies such as coarctation of the aorta, ventricular septal defect, patent ductus arteriosus, bicuspid aortic valve and Marfan's syndrome.^{3-5,8} When acquired, these lesions most often are due to syphilis and only occasionally to endocarditis. Jones and Langley¹ found forty-seven cases of sinus aneurysms reported in the medical literature up to 1949. Of these, twenty-five were congenital, seventeen were syphilitic, four originated from bacterial endocarditis and one resulted from a dissecting



Fig. 4. Photomicrograph of a section of the myocardium. Neutrophilic infiltration is seen diffusely with coalescence in one area to form a microabscess.

aneurysm of the aorta. Sawyers et al.² found a total of forty-seven congenital sinus aneurysms in the literature before 1957. Since the report of Jones and Langley, Venning⁴ has reported four cases of aortic sinus aneurysms due to bacterial endocarditis alone. Hart et al.⁶ have noted a high incidence of sinus aneurysms and endocarditis due to brucella infection.

Using the data of Jones and Langley and reviewing the literature since their report, we have discovered a total of twenty-one cases of sinus of Valsalva aneurysm due solely to bacterial endocarditis.^{1,4,6,7,9-12,14} Exact quantitation of the frequency of this occurrence is difficult due to the fact that bacterial endocarditis often complicates a congenital sinus aneurysm. Thus, it is frequently difficult to ascertain whether the endocarditis was a cause or result of the aneurysm.^{4,15}

Acquired and congenital aneurysms differ somewhat in the sinus involved and in the site of rupture. Sawyers et al. discovered that of fortynine congenital sinus aneurysms found in fortyfive patients who came to autopsy, thirty-four involved the right coronary sinus, thirteen the non-coronary and two the left sinus. Rupture had occurred in thirty-seven of the forty-five patients, with thirty-four perforations into the right atrium or right ventricle. Rupture into the pericardium was noted only once. Of the twenty-two acquired aneurysms reported by Jones and Langley, the left coronary sinus was involved in seven. Pericardial rupture occurred in two of the eleven instances of perforation.

We have found that since 1949 three cases of

bacterial endocarditis resulting in sinus aneurysm and pericardial rupture have been reported, making a total of five acquired and three congenital cases which thus terminated.^{1,2,4,6,7,13,14} To our knowledge our patient is the ninth such case.

Unruptured sinus aneurysms are asymptomatic but may be recognized by angiocardiographic technics. 16,17 Rupture into a cardiac chamber is often associated with sudden pain in the chest and dyspnea, a to-and-fro murmur near the sternum and the acute or gradual development of heart failure. The diagnosis, which may be confirmed by cardiac catheterization or angiocardiography, is of more than academic interest since the lesion has been successfully repaired surgically. 2,8

We think that the present case represents an instance of sinus of Valsalva aneurysm secondary to bacterial endocarditis rather than a congenital lesion complicated by infection. The absence of associated congenital anomalies, the presence of a history of rheumatic fever with pathologic evidence of rheumatic aortic stenosis, and the location of the aneurysm in the left

coronary sinus support this view.

Congestive heart failure rather than persisting infection is the usual cause of death related to bacterial endocarditis at this time. In the case reported here the infection due to Staph. aureus persisted despite therapy. Suppurative arteritis developed with subsequent weakening of the aortic wall, dilatation and rupture into the pericardium. It is unlikely that surgical treatment for lesions of this type will be possible because of the problem of persisting infection and difficulty in diagnosis of the aneurysm before the catastrophic pericardial rupture takes place.

SUMMARY

A case is described in which rheumatic calcific aortic stenosis was complicated by the development of endocarditis due to Staph. aureus. The infection led to the development of an aneurysm of the left aortic sinus of Valsalva with rupture into the pericardium and death.

ACKNOWLEDGMENT

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Dissociation with Double Interference Resulting in Ventricular Bigeminy*

JACOB WOLANSKY, M.D.

East Orange, New Jersey

A forty-three year old Negro man was admitted to the hospital with a clinical diagnosis of rheumatic heart disease, enlarged heart, aortic stenosis and insufficiency, congestive heart failure and multiple arrhythmias due to digitalis toxicity. Selected strips from a long lead V₁ are illustrated in Figure 1. The mechanism is shown diagrammatically by use of the customary conventions, with the time intervals in hundredths of a second.

ANALYSIS OF ELECTROCARDIOGRAMS

The dominant rhythm is interference dissociation between a sinus arrhythmia and an A-V nodal rhythm. The interatrial cycle length varies from 0.88 to 1.16 seconds. There is a slight degree of A-V nodal arrhythmia also, with internodal cycle lengths of 0.8 to 0.96 second.

In strip A, P8 and P10 effect ventricular captures which are aberrant in form. A greater degree of aberration is seen with R9, due to the fact that it is slightly more premature with respect to the preceding A-V nodal beat. Ventricular complexes similar to R9 recurred several times in other parts of this tracing. The temporal relationship of the ventricular complexes to the preceding atrial deflections was always the same, and thus ruled out the possibility that R9 might be a ventricular extrasystole.

In strip B, long pauses are present between R1 and R2, and R8 and R9, measuring 1.32 and 1.34 seconds, respectively, comparable to the internodal interval embracing the ventricular captures in strip A. These pauses in the ventricular rhythm are due to the unusual mechanism of dissociation with double interference.¹

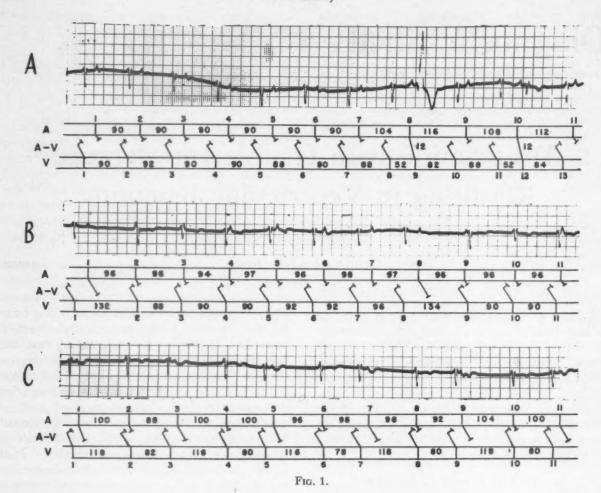
The atrial impulses P1 and P8 partially penetrate the A-V junction. They pass through the A-V node and discharge its pacemaker, but are blocked at a lower level and therefore do not result in ventricular captures. The concealed conduction of these impulses is manifested by their effect on the A-V nodal rhythm.²

In strip C, the long pauses follow every second A-V nodal beat (R1, R3, R5, R7 and R9) with a resulting bigeminal ventricular rhythm. It is to be noted that double interference occurs in strip C after R to P times which are shorter than some R to P times in strips A and B which result only in simple interference. The most likely explanation for this phenomenon is the following: It is a well-known fact that the duration of the refractory period varies inversely with the heart rate. In strip C the A-V nodal rhythm is faster than in strips A and B. Therefore, concealed conduction can occur despite the relatively short R to P time.

COMMENTS

Interference dissociation is a common disturbance of rhythm. A rare variant is called dissociation with double interference, or dissociation with ladder type of interference. Dissociation with double interference between an S-A and A-V nodal rhythm occurs when a second area of block is present below the level of the A-V nodal pacemaker. A capturing beat, although penetrating the A-V node and discharging its pacemaker, does not succeed in reaching the ventricles. The present case illustrates this mechanism. Of particular interest is the occurrence in one portion of the tracing of

^{*} From the Medical Service, V.A. Hospital, East Orange, New Jersey.



double interference after every two A-V nodal beats. Thus the dissociation with double interference gives rise to a most unusual and possibly unique type of ventricular bigeminy.

ACKNOWLEDGMENT

I wish to thank Dr. Ralph Miller, Attending in Electrocardiography and Cardiology, and Dr. H. A. Weiner, Chief, Medical Service, V.A. Hospital, East Orange, New Jersey, for their advice and criticism.

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SYMPOSIUM ON FIBRINOLYSIS



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The Development of Our Knowledge of Fibrinolysis*

PROF. F. KOLLER

Zurich, Switzerland

LTHOUGH there is no doubt that recent advances in the field of fibrinolysis, and especially in fibrinolytic therapy, are primarily due to the studies of American scientists and clinicians, the foundations for these later developments were laid in Europe. It is most interesting to see how far back they can be traced. The observation that after sudden death the blood remains uncoagulable was made 200 years ago by Morgagni,1 the well known Italian pathologist who describes this condition very clearly in his famous work "De sedibus et causis Morborum," published in 1761. A little later, in 1786, John Hunter,2 in his "Lectures on the Principles of Surgery" calls attention to the fluidity of the blood, again in cases of sudden death. The cause of this phenomenon was recognized by Denis⁸ and Zimmermann4 who observed in the early nineteenth century that fibrin of blood obtained from wet cupping redissolved in twelve to twenty-four hours.

The designation "fibrinolysis" was proposed for the first time in 1893 by Dastre, successor to Claude Bernard in the chair of physiology at the Sorbonne in Paris. It is of interest that Arthur, who discovered the role of calcium in coagulation, was working at that time with Dastre at the Sorbonne. The line of research just mentioned led to the recognition and characterization of fibrinolysis, but it was primarily descriptive.

The first attempt to act upon this phenomenon, at least *in vitro*, was made by Denys, who produced fibrinolytic, or more precisely, proteolytic activity in serum by treating it with chloroform. Later on Tagnon^{8,9} showed that this proteolytic

enzyme was fibrinolysin (plasmin) and that chloroform acted probably by eliminating an inhibitor.

Quite a different approach for experimental fibrinolysis was discovered in 1933 by Tillett and Garner¹⁰ in New York who demonstrated that human plasma clots were rapidly dissolved in the presence of filtrates of certain strains of hemolytic streptococci. The importance of this discovery can hardly be overrated. We know at present its far reaching consequences, not only for experimental research in vitro but also for investigations in vivo, in the animal as well as in man. I should like to mention here the outstanding experimental work carried out in the Sloan-Kettering Institute, particularly by Cliffton, LaDue and my former collaborator, Ruegsegger¹¹, who for the first time succeeded in dissolving coronary thrombi.

PRESENT THERAPY OF FIBRINOLYSIS

To outline briefly the bearing of these discoveries on the theory of fibrinolysis, anyone dealing with the field of blood coagulation is impressed by the similarity of these two biologic phenomena. It is true that they are in fact antagonistic: blood coagulation producing fibrin, fibrinolysis destroying fibrin. But the mechanisms by which this result is reached are strikingly similar (Fig. 1). Both systems produce finally a proteolytic enzyme: thrombin and fibrinolysin (plasmin). Thrombin splits a small particle (fibrinopeptide) from fibrinogen; fibrinolysin splits fibrin into a great many polypeptides. Besides fibrin and fibrinogen, the clotting factors v and vII are attacked as well as other proteins such as casein.

^{*} From the Krankenhaus Neumünster, Zollikerberg, Zurich, Switzerland.

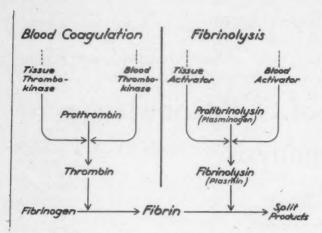


Fig. 1. Similarity of the mechanism of blood coagulation and fibrinolysis.

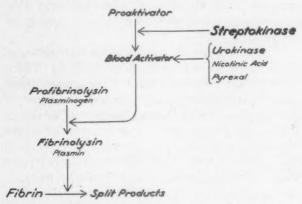


Fig. 2. Artificially induced fibrinolysis. Streptokinase acting indirectly on profibrinolysin, according to Astrup, Müllertz and Lassen.

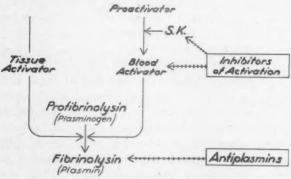


Fig. 3. Inhibitors of fibrinolysis.

Both proteolytic enzymes are derived from an inactive precursor present in plasma: thrombin from prothrombin, fibrinolysin from profibrinolysin or plasminogen, as shown by the work of Milstone, ¹² Kaplan, ^{13,14} Christensen, ¹⁵ and Kline et al. ¹⁶ The latter succeeded in obtaining plasminogen in crystallized form.

The activation of the precursor to an active enzyme can be produced in both instances by

two different mechanisms: (1) by a tissue activator (tissue thromboplastin) in the coagulation system, or (2) by a blood activator (blood thromboplastin) generated exclusively by the constituents of the blood itself.

The tissue activator of fibrinolysin has been investigated particularly by Astrup, 17,18 Müllertz, 19 Albrechtson and his co-workers20 in Copenhagen. They found considerable differences in activity in various organs; the prostate, the meninges and the thyroid were particularly active in this respect.

The formation of blood activator seems to be especially complex, a situation all too familiar to the worker in blood coagulation research. The activator appears to be derived from a proactivator postulated by Astrup, 17, 18 Müllertz, 19 Cliffton 22 and others. The existence of the latter is still controversial, Sherry and his group 23,24 denying its existence. Even if we admit that the proactivator has not yet been isolated in purified form, its existence seems at least possible; many observations, for example the variable action of streptokinase in different species, would be hard to explain without it.

It is evident from the scheme represented in Figure 2 that artificial fibrinolysis can be induced in two ways: (1) by injection of streptokinase in the blood stream which initiates the whole mechanism, or (2) by injection of the end product of the entire process: fibrinolysin or plasmin. There is a third possibility, the injection of urokinase, an active principle of the urine closely related to the blood activator, which was used by Celander, 25 Ambrus 26 and their co-workers.

Of interest is that several body fluids, such as milk, tears and urine, contain a fibrinolytic activator apparently without a corresponding inhibitor which protects the organism, at least to a certain degree, against the formation of coagula obstructing the excretory ducts.

FIBRINOLYSIN INHIBITORS

A biologic phenomenon of the importance of fibrinolysis is unimaginable without the existence of a counter regulation, i.e., without an inhibitory mechanism. Several inhibitors have been postulated and described by Jacobsson, ²⁷ Shulman, ²⁸ Guest, ²⁹ Ferguson ³⁰ and Ratnoff et al.; ³¹ at least two antiplasmins directed against the end product plasmin, an inhibitor of the blood activator and, most important, an inhibitor of streptokinase (antistreptokinase) (Fig. 3). Fortunately, these inhibitors are not

ER III III

Heparin

TABLE 1 Antagonists

: Protamin

Dicumarin: Vitamin K₁
Fibrinolysin: E-Aminocaproic acid

only unwelcome complications during treatment but, owing to the work of Ablondi,32 we are today in the position of voluntarily creating inhibitory effects. These authors found that E-aminocaproic acid, a most harmless substance chemically released to lysine and leucine, produces a marked inhibitory action on plasmin as well as on its activation. The availability of this antagonist has greatly enhanced the safety of fibrinolytic therapy, as protamin and vitamin K1 have done it for anticoagulant therapy (Table 1). Because of the inhibitors mentioned, the dosage problem has proved to be more complex than expected.33 The individual variations are much more pronounced than in anticoagulant therapy.

Conclusions

Many questions are still controversial and will probably be answered in this Symposium. For example, how long should a fibrinolytic state be maintained in order to be efficient in dissolving venous and arterial thrombi? At what moment must anticoagulant treatment be started in order to prevent recurrences of thrombosis?

Fibrinolysis is in many respects still a young field. It may be a surprise to learn that fibrinolytic therapy, although virtually started in 1933 with Tillet's discovery, is still in a preliminary stage today, twenty-seven years later! However, the purification of heparin needed twenty years and only thirty years after its discovery was it used on a large scale. Therefore, there is no reason to be concerned about the relatively slow advance of our knowledge in fibrinolytic therapy and the many difficulties encountered in its realization. Our goal, the complete clinical and anatomic restitution of thromboembolic disease, is so high that no effort in this direction can be too difficult.

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Spontaneous Fibrinolysis*

GEORGE R. FEARNLEY, M.D., M.R.C.P.

Gloucestershire, England

While induced fibrinolysis is well recognised as a therapeutic measure of promise, it is not so widely appreciated that freshly obtained blood has spontaneous fibrinolytic activity. This activity is slight compared to that produced by streptokinase, but its smallness as revealed by the highly artificial conditions of the test tube does not prove that it is physiologically negligible.

Far from being a chance pathologic finding, spontaneous fibrinolytic activity seems to be a property of the blood of all healthy people, and I would like to present some aspects of its behavior and measurement. In 1937 Macfarlane1 reported fibrinolytic activity in the diluted plasma of patients after surgical operations. Later, with Biggs and Pilling,2 he found by the same technic that violent exercise or injection of epinephrine would induce fibrinolytic activity in healthy volunteer subjects. No such activity was demonstrable by these workers in the blood of people who did not undergo stress. With Revill and Tweed, I3 repeated Macfarlane's experiments with epinephrine and obtained variable results. This variability was explained when we discovered that fibrinolytic activity induced by administration of epinephrine is labile in fluid blood and plasma, can be preserved by chilling and is stabilized by clot formation. Tweed and I,4 using a low temture technic between obtaining blood and setting up the tests, were then able to show that, contrary to Macfarlane's finding, fibrinolytic activity is a property of the blood of healthy people who have not undergone stress. Assuming that this is due to the presence of factor in blood other than plasmin, we have given it the non-specific name of labile fibrinolytic component. 5 Such a factor, to my knowledge, has not been isolated, but granting its existence it seems likely to be an activator of plasminogen perhaps analogous to or even identical with that known to be present in urine and other body fluids.

MEASUREMENT OF FIBRINOLYSIS ACTIVITY

Current methods of measurement of spontaneous fibrinolysis are crude and depend on lysis of the subject's own fibrin. All require dilution of blood or plasma, presumably to dilute out inhibitor. Nevertheless, provided the subject's fibrinolytic activity is great enough, we have found that clots made of undiluted blood with thrombin in the absence of calcium will lyse within twelve to twenty-four hours' incubation. This suggests that we are not dealing with a dilution artifact. The presence of calcium is extremely inhibitory to spontaneous fibrinolysis and it seems unlikely that this is entirely due to the fact that calcium fibrin is tougher than thrombin fibrin.

Present methods give an over-all measure of fibrinolytic activity in dilute blood or plasma and with the exception of the euglobulin lysis time which probably measures uninhibited activity, are affected by at least three variables, namely, amount of activator, amount of inhibitor, and amount of fibrinogen. The rate of

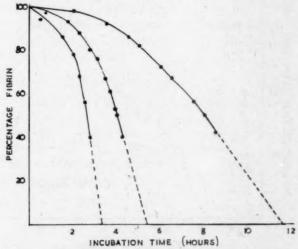


Fig. 1. Percentage of disappearance of fibrin from 10 ml. volume clots made with thrombin of plasma from three healthy subjects diluted 1:10 in phosphate buffer, pH 7.4 and incubated at 37 °c.

^{*} From the Gloucestershire Royal Hospital, Gloucestershire, England.

INCUBATION TIME (MINUTES)

2

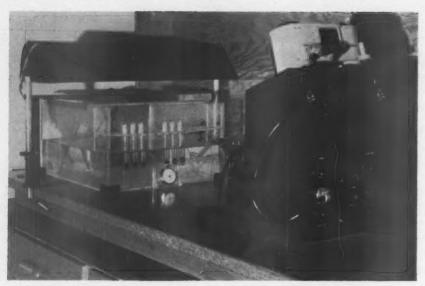


Fig. 2. Apparatus for photographic measurement of clot lysis time.

disappearance of fibrin is not linear, but accelerates to a uniform velocity. Figure 1 shows the percentage of disappearance of fibrin in clots of 10 ml. volume made of plasma diluted 1:10 in phosphate buffer. The points are the means of duplicate determinations. The measurement was inaccurate after 70 per cent of the fibrin disappeared but the lines could be extrapolated to complete lysis judged visually. Because the relation is not linear we have found determination of percentage disappearance of

With incubation the clots retract to a uniform size so that the progress of lysis can be easily photographed⁸ (Fig. 2). The method is reasonably accurate. In a series of 100 consecutive determinations in duplicate, with exposures being made at thirty-minute intervals, the results were as follows: in fifty-two the lysis times were identical; in twenty-three there was a half-hour difference; in eighteen a difference of one hour; in two a difference of one and a half hours, in three a difference of two hours; and in two a difference of three hours. This gives a mean error of 8 per cent calculated from the actual lysis times. FACTORS AFFECTING LYSIS TIME PA BLOOD RESTING BLOOD

(LYSIS TIME HOURS)

Fig. 3. Reduction of fibrinolytic activity in citrated blood incubated at 37 °c. from zero to one hour, the standard test being set up at ten-minute intervals on resting and postepinephrine blood samples of a male subject. Solid line=resting blood; broken line=postepinephrine blood (P.A.).

8

10

12

6

Epinephrine: Figure 3 shows the lability of spontaneous fibrinolytic activity. Blood was obtained and citrated. A test was set up immediately, giving a lysis time of three and a half hours, and the remainder of the citrated blood was placed in the waterbath at 37°C. At intervals, further tests were set up on this blood. One hour's incubation of the fluid blood resulted in the initial lysis time of three and a half hours lengthening to thirteen hours. The same subject was given a subcutaneous injection of 0.25 mg. epinephrine and the same procedure carried out. After epinephrine

fibrin an unsatisfactory measure of spontaneous

fibrinolysis, and we prefer to measure the time

of complete lysis of a dilute blood clot. The

test consists of the lysis time at 37°c. of a clot

made with thrombin of a 1:10 dilution of blood

in phosphate buffer pH 7.4, molarity 0.07.7

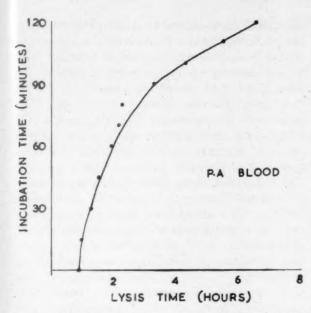


Fig. 4. Reduction of fibrinolytic activity in citrated blood postepinephrine incubated from zero to two hours, the standard test being set up at tento fifteenminute intervals. The subject was the same as in Figure 3.

administration fibrinolytic activity increased greatly, the initial lysis time being forty minutes. With one hour's incubation this lengthened to two hours. Figure 4 shows the same experiment on postepinephrine blood from the same subject on another occasion, the incubation period being two hours. The greater prolongation of lysis time occurs during the second hour and brings out more clearly that postepinephrine fibrinolytic activity shows the same lability as does spontaneous activity from the subject who has not undergone stress.

Temperature: We have recently found that not only is fibrinolytic activity labile in fluid blood but also that the temperature of fibrin formation profoundly affects lysis time. Table I shows the lysis times with different temperatures of fibrin formation. It is evidently necessary to follow a rigid low temperature technic between obtaining blood and incubating the clots to obtain reproducible results.

Molarity of Diluent: Another factor which affects the velocity of fibrinolysis, as pointed out by Buckell and Truscott⁹ and also by Weiner,¹⁰ is the molarity of the diluent. We have confirmed this by varying the molarity of our phosphate buffer, and this effect of molarity would seem to explain the apparently inhibitory effect of normal saline reported by Ferguson and myself.⁶

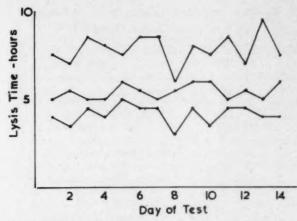


Fig. 5. Blood clot lysis times of three healthy male subjects at 6 P.M. daily.

NORMAL FIBRINOLYTIC ACTIVITY AND DIURNAL BEHAVIOR

During the past year we have made determinations on both healthy people and patients with various diseases in the hospital. With this method the lysis times of most people, irrespective of age, sex and time of day, lie between two and ten hours, with a mode of five hours. For many people the lysis time measured at the same time of day lies within fairly narrow limits. Figure 5 shows the lysis times of three healthy men measured daily at 6 P.M. for fourteen days. In two there is little variation but the third showed greater fluctuation. Three of a group of four young nurses we studied, however, showed greater variability which we have not been able to relate clearly to the menstrual cycle. Hospitalized patients of both sexes under standard conditions who are not acutely ill also have constant lysis times.

TABLE I

Effect of Temperature of Fibrin Formation on Lysis Time

	Clotting Temperature*					
Subject	0°R	0°В	R.T.	37°B		
1	4 hr., 42 min.	4 hr., 58 min.	6 hr., 35 min.	7 hr., 5 min.		
2	3 hr., 35 mip.	4 hr., 4 min.	5 hr., 17 min.	5 hr., 38 min.		
3	4 hr., 30 min.	4 hr., 40 min.	5 hr., 35 min.	7 hr., 25 min.		

^{*} The following abbreviations are used: 0°R = set up at 0°c., and kept at this temperature for thirty minutes before incubation; 0°B = set up at 0°c., and incubated immediately; R.T. = set up at room temperature (22°c.) and incubated immediately; 37°R = set up at 37°c. in the waterbath.

TABLE II
Lysis Time (hr.) of Inpatients

Women			Men		
Age (yr.)	A.M.	P.M.	Age (yr.)	A.M.	P.M.
37	4	2	44	6	4
20	5	3.5	48	7	
32	6.5	5	46	7	3.5
61	5	4	59	4	3
37	4.5	3	47	7.5	6
56	7	5	52	6	5
62	7.5	7	60	7.5	7
44	4	4	68	12	11.5
69	5	5	37	4	4
35	6	6	57	6	6
57	11.5	11.5	50	5.5	5.5
49	2.5	3	64	5.5	6
42	7	7.5	48	4	5
46	5.5	6	42	3.5	4.5
64	2.5	3	62	3.5	5.5
40	5	6	54	7 5	8
70	3	4.5	45		7
60	3.5	6	59	4	7
58	7 -	10	55	2.5	5
40	4.5	8	49	4	8
Mean 49.5	5.3	5.5	52.3	5.6	5.8
S.D. 11.1	2.03	2.25	7.9	2.06	1.7

Diurnal Variations: In 1957 we reported a diurnal variation of fibrinolysis, the lysis times being longer at 4 A.M. than at 4 P.M., and diurnal variability has been confirmed by two other groups of workers.11,12 Our subjects were young nurses, and now that we have wider experience in spontaneous fibrinolytic activity, we would say that the differences were noteworthy in only half the nurses studied. A study of hospital inpatients has shown no consistency of diurnal variation. The morning specimens were, however, obtained at 8 A.M. instead of 4 A.M. Table II gives the lysis times of forty inpatients whose blood was obtained at 8 A.M. in a fasting state, and at 5 to 6 P.M. on the same day. Patients with infectious, neoplastic and arteriosclerotic diseases were excluded. It can be seen that in some the lysis times were longer in the morning than in the evening but that in others the reverse occurred. Many showed differences which are hardly significant. We have found, however, some patients with very low activity in the morning but faster activity at 6 P.M., and this has been confirmed by repeated determination. It appears that blood

fibrinolytic activity varies from person to person with significant variations in some people with the time of day but not in others. This means that for comparable measurements the same time of day should be chosen.

Occlusive Vascular Disease: Using the same method of measurement, Nestel13 has reported a highly significant difference between the lysis times of patients with intermittent claudication and a group of healthy control subjects. The lysis times of the patients with claudication were much longer than those of the control subjects, and there was little overlap. Our data on patients with occlusive vascular disease of the heart and limbs are far from complete, and it would be premature to make definite statements. However, the majority of the patients we have studied so far, particularly those with coronary artery disease, have lysis times no longer than those of the control subjects. A proportion, however (it is too early to say what percentage they constitute), do seem to have very low fibrinolytic activity.

Meals: Spontaneous fibrinolytic activity in the blood is increased by exercise and has been reported to be diminished by fatty meals, ^{12,14–16} but Hougie¹⁷ has been unable to confirm this. We have been unable to relate fibrinolytic activity to the fat content of the meals preceding the tests in our subjects, nor have we been able, using this method of measurement in a small number of experiments, to produce any significant change of lysis time by inducing alimentary lipemia. It is quite possible that spontaneous fibrinolysis is reduced in some people by fat feeding, but not in others.

COMMENTS

Is the spontaneous fibrinolytic activity of blood, requiring as it does considerable dilution for its demonstration, likely to be of any importance? I believe that it may be for several reasons. First, the lysis of undiluted blood clots of healthy subjects with enough fibrinolytic activity suggests that the labile fibrinolytic factor is not a dilution artifact, and is present as such in blood as obtained from the body. Second, I showed some years ago that labile fibrinolytic component is absorbable by plasma clot, and that clots made from non-fibrinolytic plasma after being steeped in fibrinolytic plasma and washed, show lysis on incubation.18 It is possible, therefore, that concentration of labile fibrinolytic component by adsorption on sites of deposited fibrin in vivo could result in greater

and more effective lysis than is observed in the test tube. Conversely, the inhibition imposed by calcium at the time of clotting could ensure the stability of occlusive thrombi, that is those to which circulating blood has no access. This does not take into account the evidence of McFadzean and his associates19-21 that blood fibrinolytic activity derives from the blood vessels. It is possible that the activity measurable in blood may be a pale but accurate reflection of a far greater activity in the vessels themselves. Finally, from its behavior, its reproducibility, and to a great extent its predictability, spontaneous fibrinolysis seems to deserve recognition as a biologic phenomenon. As a crude measurement it is comparable to the clotting time of blood, of which it may be conjectured to be the antithesis.

If we are right in postulating that spontaneous fibrinolysis indicates the existence of a functioning homeostatic system opposed to coagulation, then the possibility exists of influencing its behavior pharmacologically. Such an approach implies the hope of prevention rather than treatment, and differs fundamentally from the concept of direct stimulation of the fibrinolytic system.

SUMMARY

Freshly obtained blood has spontaneous fibrinolytic activity, which is labile in fluid blood and plasma, can be preserved by chilling, and is stabilized by clot formation.

Its measurement and behavior are discussed.

Reasons are advanced for believing that spontaneous fibrinolytic activity indicates the existence of a functioning system opposed to coagulation, which might be enhanced pharmacologically rather than by direct stimulation.

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DISCUSSION OF PAPERS BY DRS. KOLLER AND FEARNLEY

DR. ERWIN DEUTSCH (Vienna, Austria): We studied the influence of fatty meals on the dissolution of clots. We used the euglobulin lysis time and the streptokinase resistance test and found that the euglobulin lysis time was one or two hours longer in the lipemic blood sample than in the fasting one. In the same way a greater amount of streptokinase was necessary to obtain the lysis time of ten minutes in the lipemic blood sample than in the fasting one.

DR. F. K. Beller (Stuttgart, Germany): Many reports concerning fibrinolysis deal with estimation

methods which involve streptokinase as the activator by addition to plasma or euglobulins. We pointed out that these methods are unsuitable for quantitative estimations of plasminogen or plasmin activities.

Fletcher has already demonstrated that a quantitative conversion from plasminogen to plasmin occurs only at low precursor concentrations. Our experimental series using serial plasma dilutions gave the same results. At a plasminogen concentration of approximately 5 to 10 units per ml. the curve takes an angular shape passing over to a nearly horizontal line. By schematically dividing this curve into two segments it was obvious that the usual estimations by means of dilution series are related to the horizontal segment of the curve which does not represent the respective real enzyme activity. In accordance with Astrup we assume that this kind of assay with streptokinase reveals the proactivator or activator function rather than the plasmin or plasminogen activity.

The investigations of inhibitory agents are more complicated. In our opinion no clinical method exists which gives accurate estimations of inhibitory substances in blood. The generally used methods of obtaining so-called inhibitor-free plasmin by diluting plasma or salting out, in order to investigate its influence on undiluted plasma after certain incubation periods, are not suitable.

DR. JULIAN L. AMBRUS (Buffalo, New York): Does Dr. Fearnley think that variations in fibrinogen concentration in the blood of patients he measures may influence his method?

DR. MASON GUEST (Galveston, Texas): Dr. Fearnley's method has been an important contribution. The sensitivity of this method is increased by rapidly reducing the pH of the blood to 6.4 immediately after it is drawn. Lysis times are reduced to from fifteen minutes to one hour when this technic is used. In our modification, phosphate buffer replaces the veronal buffer that Dr. Fearnley originally suggested.

DR. F. KOLLER (Zurich, Switzerland): May I recall that Dastre demonstrated clearly that normal human blood has fibrinolytic activity?

Dr. A. L. Copley (New York, New York): I reported findings of anticoagulant action of fibrin-coated or so-called fibrinized surface (Copley, A. L. Proceedings of the Seventh European Society of Hematology, London, 1959. Basel, 1960. S. Karger). In preliminary observations on heat-treated bovine fibrin surface, made from Armour's fraction I, increased fibrinolytic activity has been found by the euglobulin fibrinolysis test. Studies on fibrinolytic activity in non-altered fibrinized tubes are now in progress. If fibrinolysis should prove to be increased in systems contacting non-altered fibrin surface, any considerations of extracorporeal fibrinolysis will involve the nature of the contacting surface. In all extracorporeal studies reported on fibrinolysis, any contacting surface

employed has been foreign to blood. Investigations on fibrinolysis might have quite different results if the system under study is in contact with a fibrin surface.

DR. NILS BANG (St. Louis, Missouri): Regarding Dr. Koller's finding of complete disappearance of fibrinogen in the course of infusion of streptokinase, if this is a true observation it must constitute a serious objection to this type of therapeutic management. The question is whether the thromboelastographic technic which Dr. Koller has used for estimation of fibrinogen gives the correct picture of the actual fibrinogen content at any given time. As shown by Johnson and by Sherry's group, infusions of streptokinase in doses similar to those indicated by Dr. Koller will cause a drop in fibrinogen but only of about 33 to 50 per cent of the pretreatment values. However, as was pointed out first by Niwiarowsky and Kowalsky and by Fletcher, another important factor is the presence of breakdown products from fibrinogen in plasma. These breakdown products interfere with the second and third steps of fibrin polymerization leading to a striking coagulation defect which may account for the observed thromboelastographic tracings. The presence of these products of fibrinogen split are probably of the greatest importance in our evaluation of the results of therapeutic management of patients treated with streptokinase.

Dr. Fearnley (closing): The question of alimentary lipemia affecting fibrinolysis might be one of method. It is interesting that Dr. Deutsch finds a constant effect when the euglobulin lysis time is used since we do not with our method. We found that about 20 per cent of our patients with occlusive vascular disease show low fibrinolytic activity. It may be that the majority of our patients are in the older age group and that if we studied younger people, we would find a higher percentage. I am interested to learn that the arteriovenous difference we reported has been confirmed in a larger series of cases. I have believed for some time that perhaps we should measure fibrinolysis on the arterial side rather than on the venous side of the system. Regarding a basic level for measuring fibrinolytic activity, I agree this is difficult. As has been suggested (BILLIMORIA, J. D., DRYSDALE, J., JAMES, D. C. O. and MAGLAGEN, N. F. Lancet, 2: 471, 1959), one should almost prepare the patient as for a basal metabolic rate test, but when a large number of determinations are taken in an ordinary hospital ward, this is impossible. We make most of our measurements at 8 A.M. on the fasting patient, which seems to be the best time for reproducible results from day to day.

To reply to Dr. Ambrus, I am sure that variations in the amounts of fibrinogen influence the results. We are now measuring fibrinogen levels in patients with occlusive vascular disease. Dr. Guest's work on decreasing the lysis time is an important contribution which may solve the problem of the rather long clot lysis time we have to obtain by photography. Does the ratio between the lysis time at pH 6.4 to that at 7.4

remain constant? Lackner and I found some years ago (Fearnley, G. R. and Lackner, H. Brit. J. Haemat., 1: 189, 1955) that clots made by recalcification as compared with clots made with thrombin give pH optima for fibrinolysis which are quite different. The pH optimum we found at that time for a clot made with thrombin was 7.2 whereas when recalcification was used it was 8.6.

Dr. Koller (closing): I agree with Dr. Bang that the thromboelastogram is a crude method for determining fibrinogen. We determined fibrinogens with other methods and found markedly decreased values by, for instance, 50 mg. per cent or even less, but not zero. Despite this the thromboelastogram is valuable because it indicates that physiologic fibrinogen with normal polymerization is really lacking.



Physiologic and Pathologic Effects of Increased Fibrinolytic Activity in Man

With Notes on the Effects of Exercise and Certain Inhibitors on Fibrinolysis*

OSCAR D. RATNOFF, M.D.† and VIRGINIA H. DONALDSON, M.D.

Cleveland Ohio

LTHOUGH phenomena attributable to the potent proteolytic properties of blood have now been studied for almost two hundred years, their physiologic significance remains an enigma. In his perceptive autobiography, Walter Cannon1 wrote, "My first article of belief is based on the observation, almost universally confirmed in present knowledge, that what happens in our bodies is directed toward a useful end." Expressed in this manner, Cannon's teleologic view is one which many persons find distasteful. Still, as he pointed out, teleologic reasoning can be of the greatest help in the elucidation of physiologic mechanisms. Von Bruecke put it this way, "Teleology is like a scientist's mistress. He may be unable to live without her, yet he is ashamed to show himself with her in public." But when we come to search for a physiologic meaning to the proteolytic properties of plasma we are confronted with an accumulation of data which are still too bewildering to us to allow for any meaningful interpretation.

PHYSIOLOGIC ACTIVITY OF PLASMIN

The bulk of the proteolytic activity of plasma resides in the enzyme or enzymes commonly called plasmin, or, less happily, fibrinolysin. When active, this enzyme is capable of digesting a large number of substrates, some found in the body and some which it is unlikely to meet in nature. Thus, plasmin can digest fibrinogen, fibrin, proaccelerin, antihemophilic factor, prothrombin, Christmas factor, 2 the first component

of complement,³ ACTH, glucagon and somatotropin,⁴ all of which may be present in the circulating plasma. In addition, it readily digests casein,⁵ gelatin,⁵ denatured hemoglobin,⁶ betalactoglobulin,⁷ azocoll (a preparation of collagen coupled to an azo dye),⁸ and certain synthetic esters of arginine and lysine.⁹ This is a bewildering list and makes one wonder which, if any, of these substrates is most likely to be attacked in nature.

The proteolytic activity available in human plasma is of considerable magnitude. For example, the amount of plasmin in just one milliliter of normal plasma, suitably activated by streptokinase, will digest an amount of fibrinogen equivalent to that in the entire volume of circulating plasma within an hour or less. ‡ The teleologist would immediately assume that plasma must contain inhibitors which can neutralize the effects of any plasmin which might be activated within the blood stream. Indeed, such inhibitors were described early in this century by Delezenne and Pozerski. ¹¹ The inhibitory ac-

‡ The type of reasoning on which this calculation is based may well be erroneous. The assessment of the fibrinolytic property of plasma is based on experiments in which plasma is serially diluted with buffered saline, and then activated maximally with streptokinase. The activated plasmin is then added to bovine fibrinogen and the mixture is clotted with thrombin. Conceivably, the plasmin activates bovine plasminogen, adsorbed to the fibrin, and this in turn digests the fibrin clot. If this is the case, an estimation of the fibrinolytic activity of human plasmin would be impossible.

^{*} From the Department of Medicine, Western Reserve University School of Medicine, and the Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio. Previously unpublished studies described in this report were supported by Research Grant H1661 from the National Heart Institute, National Institutes of Health, U. S. Public Health Service, and by grants from the Cleveland Area Heart Society and the American Heart Association.

[†] Career Investigator, the American Heart Association.

tivity of plasma is probably attributable to more than one substance, ^{12,18} and can be differentiated from the inhibitors of trypsin and chymotrypsin which are also found in normal blood. ¹⁴

Plasminogen: There is overwhelming evidence that plasmin exists in the blood in the form of an inactive precursor, variously called plasminogen or profibrinolysin. Plasminogen can be converted to plasmin in a number of different ways. Although the suggestion had been made earlier, Delezenne and Pozerski¹¹ first clearly demonstrated that proteolytic activity appeared when plasma was treated with chloroform. Since then, other agents have been described which will activate plasminogen. These include, besides chloroform, acetone, 15 alcohol, 15 streptokinase (a principle found in cultures of beta hemolytic streptococci) 16 and staphylokinase (a substance found in cultures of staphylococci). 17 In addition, plasminogen may be activated by substances present in human milk,18 tears,19 saliva, 20 seminal fluid, 21 and of greatest interest at the moment, urine.22 None of the substances mentioned seems likely to explain how plasminogen may become active within the body. It is possible that the agents found in the bodily secretions may be the excretory products of activators circulating within the blood stream.

Plasminogen may also be activated by the addition of small amounts of tissue derived from a variety of organs.^{23,24} Perhaps under suitable conditions this tissue activator comes in contact with the circulating blood and in this way generates proteolytic activity. Trypsin²⁵ can activate plasminogen too, and it is possible that this pancreatic enzyme may inadvertently enter the blood stream and induce the formation of plasmin. But, evidence for this is not available. Certainly, plasmin and trypsin are easily differentiated enzymes

easily differentiated enzymes.

Spontaneous Activation of Plasmin: Whether any of the activators found in the tissues or secretions of the body play a part in the development of proteolytic activity in circulating blood is an important but unsettled question. There is, however, no doubt that when human blood is withdrawn from the body, allowed to clot and incubated at body temperature, the clot may then dissolve. This phenomenon of fibrinolysis is more readily demonstrated in clots prepared from cell-poor plasma than from whole blood. Presumably, then, the plasma itself possesses some way by which its fibrinolytic enzymes become active.

How this spontaneous activation of plasmin

comes about is unknown. It is known that fibrinolysis, the solution of a fibrin clot, is hastened by diluting the plasma with normal saline solution before the clot is formed.28 Fibrinolysis is also more rapid in clots formed from a crude euglobulin fraction of plasma. 23,29 This euglobulin fraction is easily separated from the rest of the plasma and so lends itself to the study of fibrinolytic phenomena. However, the rate of lysis of the clotted euglobulin fraction may not parallel that of clotted plasma. How it is that diluting the plasma with saline solution or separating the euglobulin fraction accelerates fibrinolysis is not certain. One ready explanation is that both procedures decrease the inhibitory activity of plasma against active plasmin, but the data which support this view are not entirely satisfactory.

None of these observations explains how it is that clotted plasma may liquefy. A considerable literature, ably reviewed by Sherry, Fletcher and Alkjaersig, 30 suggests that under certain circumstances the plasma may contain an activator of plasminogen. The chemical nature and source of this activator are uncertain. In this view, the activator induces the conversion of plasminogen to plasmin and the active enzyme then digests the fibrin clot. However, direct evidence that an activator of plasminogen actually exists in human plasma is meager. A second hypothesis suggests that plasminogen becomes active only when the inhibitors of plasmin deteriorate.³¹ It is known that plasminogen, freed of inhibitors, is gradually converted to plasmin by an autocatalytic process. 32,33 When plasma is incubated, its inhibitory activity against plasmin gradually deteriorates. When this inhibitory activity falls to a critical level, the plasminogen is converted to plasmin, possibly by an autocatalytic reaction. Of course, these two hypotheses are not incompatible, for it may be that any activator of plasminogen would become more effective when the inhibitors of the enzyme deteriorate.

Effect of Streptokinase: Evidence also exists that under some conditions the clotting process itself may favor the development of fibrinolytic activity. 34,35 The effect of clotting is most clearly demonstrated in studies of the action of streptokinase on plasma. 36 When an appropriate amount of active plasmin is added to plasma, it digests the plasma's fibrinogen to the point at which it can no longer be clotted to fibrin. The same amount of enzyme readily liquefies a fibrin clot. Active plasmin seems to

digest fibrinogen and fibrin with equal avidity.37 A different situation exists when we study the activation of plasminogen by streptokinase. When streptokinase is added to plasma, the plasma's fibrinogen is slowly digested to the point at which it is no longer coagulable. In contrast, if the mixture of streptokinase and plasma is clotted, the fibrin which forms lyses rapidly. These experiments seem to show that the formation of effective amounts of plasmin occurs at a faster rate in the presence of a clot than in unclotted plasma. One explanation for this observation is that plasminogen and streptokinase are both readily adsorbed onto a fibrin clot, particularly if they are both present when the clot forms. Plasminogen and streptokinase then form plasmin in intimate contact with its substrate fibrin, unhampered by the ininhibitors circulating in the plasma.38 Similarly, when plasma is incubated with plasmin, its antihemophilic factor, Christmas factor and proaccelerin are rapidly inactivated. On the other hand, when plasma is incubated with streptokinase under identical conditions, only proaccelerin activity is lost.2 Again, activation of plasminogen by streptokinase seems incomplete in unclotted plasma.

The same distinction between the effect of streptokinase on fibrinogen and fibrin can be seen in experimental animals. Johnson and Tillett³⁹ showed that the intravenous injection of streptokinase into laboratory animals was followed by lysis of intravascular thrombi. On the other hand, the same amount of streptokinase produced relatively little change in the concentration of fibrinogen in the animal's plasma. In their experiments, the plasmin produced by injecting streptokinase had a greater effect on the fibrin of an intravascular clot than

on circulating, unclotted fibrinogen.

Effects of Stress: When clots formed from normal plasma are incubated at body temperature, spontaneous lysis usually occurs at a slow rate. Ordinarily, normal clotted plasma does not liquefy for several days, and sometimes the clots may not dissolve for weeks. Fibrinolysis may be much more rapid when the plasma is obtained from an individual undergoing some form of stress. Under these circumstances, lysis may occur within a matter of hours or within a day or two. Thus exercise, 40 fear, 41 the stresses of surgery 41 and childbirth, 42,48 electroconvulsive therapy, 44 and the injection of pyrogenic substances 45,46 all accelerate fibrinolysis in clotted plasma. What causes the rapid lysis after these stresses is not

known. It is conceivably related to the release within the vessels of an activating substance. Biggs⁴⁰ demonstrated that the intravenous injection of adrenalin induced a transient increase in the rate of fibrinolysis. More recently, McFadzean and his associates^{47–40} showed that a stimulus for rapid fibrinolysis may arise locally within a blood vessel. They occluded an extremity with a tourniquet and observed that ischemia or the injection of adrenalin, pituitrin, histamine, serotonin or acetylcholine into or alongside of a blood vessel induced rapid fibrinolysis in blood drawn from the vessel.

The physiologic meaning of the fibrinolytic response to these various stresses is obscure. The increase in the rate of fibrinolysis is only rarely dramatic, and often requires special and rather artificial techniques for its demonstration. It is hard for the teleologist to understand what useful purpose is served by a device which will lead to the solution of the very clots which staunch bleeding from a wound. Certainly, no hemorrhagic symptoms accompany the increased lytic activity and hemostasis at the site of injury seems unimpaired in individuals who undergo stress.

Exercise: It is of interest that the increased rate of fibrinolysis, as measured in a test tube, is not accompanied by a fall in the concentration of fibrinogen in the circulating plasma. A group of twelve healthy young men exercised by running at 3.4 miles per hour on a treadmill set at an incline of 10 degrees for ten minutes.* Each man carried a forty pound pack on his back. Blood was drawn twice before the start of the exercise, just after the exercise stopped and after a twenty-minute rest. Prior to the period of exercise, the lysis time of clotted plasma ranged from about forty hours to seven days. Immediately after the subjects stopped running, the clot lysis time fell to less than eighteen hours in every case; in one subject the clot dissolved in less than five hours. The clot lysis time nearly always remained rapid after the subjects had rested for twenty minutes. As we expected, therefore, exercise accelerated clot lysis. We also measured the concentration of fibringen in the same specimens of plasma. No significant change in the concentration of fibrinogen occurred as a result of the exercise, nor were changes noted in the concentration of any other clotting factors. The same results were obtained

^{*} Drs. T H. Holland, R. Seibert, S. Inkley, T. W. Moir and H. K. Hellerstein participated in the performance of these studies.

in a study of women after childbirth. Although fibrinolysis was more rapid after childbirth, no appreciable change occurred in the concentration of fibrinogen in plasma.⁴² These observations again imply that the formation of active plasmin occurs more readily in the presence of a clot. The lysis of a clot is accelerated, but the concentration of fibrinogen in unclotted plasma is unchanged.

FIBRINOLYSIS IN PATHOLOGIC STATES

Sudden Death: Excessively rapid lysis of blood clots also occurs in certain disease states. John Hunter was probably the first to describe pathologically rapid fibrinolysis, although he looked at the phenomenon in a different way than we do. Hunter noted that the blood of individuals who had died suddenly was liquid and did not clot. Presumably, after sudden death the blood clots rapidly and the fibrin then dissolves. Everyone knows how the Russian physicians have taken advantage of this phenomenon, harvesting the blood of individuals who have died suddenly to use for blood transfusion. 50

Hepatic Disease: In a number of different diseases, excessive fibrinolysis may also be noted in blood drawn during life. Goodpasture,26 in 1914, noted that the clotted blood of four patients with cirrhosis of the liver dissolved within a few hours when incubated at 37°c. On the contrary, when the unclotted plasma of these patients was incubated for twenty-four hours, the concentration of fibrinogen did not decrease. Again, fibrinolysis seemed faster than the digestion of fibrinogen. Excessively rapid fibrinolysis in the clotted plasma of patients with chronic disease of the liver has been described repeatedly since Goodpasture's initial report. In one series, the clotted plasma of normal subjects did not dissolve during the first forty-eight hours of incubation.25,27 On the other hand, the plasma of almost every patient with cirrhosis dissolved in forty-eight hours or less. One immediately wonders to what extent the increased fibrinolytic activity may contribute to the bleeding tendency of patients with cirrhosis. It seems unlikely that the amount of active plasmin in the circulation could be responsible for the coagulative abnormalities characteristic of hepatic disease. Indeed, other explanations for these abnormalities seem more reasonable. 81 However, one can easily imagine that in a patient with cirrhosis the clot which staunches bleeding from a varix might dissolve prematurely, leading to fresh hemorrhage. To our knowledge, this speculation is unsupported by any direct evidence.

Rapid fibrinolysis of the moderate degree seen in patients with cirrhosis has also been described in association with many different diseases. Thus rapid clot lysis has been reported in cases of intravascular hemolysis, ^{27,52} recent thrombotic or embolic disease, ²⁸ and in many other disorders. ^{25,27,41,53-56} In most instances rapid lysis reflected hepatic damage or a period of anxiety, stress or shock and not the particular disease which afflicted the patient. Again, as in the case of cirrhosis, it is difficult to attribute a bleeding tendency to the action of plasmin.

Fibrinolytic Purpura: Finally, a rare and unusual patient exists in whom the process of fibrinolysis is so violent and rapid that it appears to be responsible for a hemorrhagic state. 66-64 This syndrome has an emotional appeal which has led to the publication of innumerable cases in which bleeding has been attributed to fibrinolysis. In the overwhelming majority, critical analysis of the published data fails to support the diagnosis of fibrinolytic purpura. It may be worthwhile to mention two of several pitfalls in diagnosis. First, there are many clinical conditions in which the concentration of fibrinogen in the circulating plasma is diminished. This may be the case, for example, in certain of the hemorrhagic disorders of pregnancy or parturition, in a variety of neoplasms, particularly carcinoma of the prostate, or in such acute disorders as transfusion reactions or purpura fulminans. When blood is drawn from a patient with hypofibrinogenemia, the clot which forms appears at first to be of normal size. However, it quickly retracts to a tiny ball of fibrin from which most of the red cells are extruded. This ball is easily lost among the red cells and it is tempting but erroneous to assume that fibrinolysis has occurred. Even more subtle traps may ensnare us. If the concentration of fibrinogen in the circulating plasma is low, then the amount of fibrin in clotted plasma will be low. Since the substrate used to test for fibrinolytic activity is often the patient's own fibrin, it is no wonder that a small amount of fibrin is digested more quickly than a large amount. We do not mean to imply that we can offer a satisfactory alternative explanation for the pathogenesis of hypofibrinogenemia in most cases. Still, a faulty explanation may not be more valuable than no explanation at all.

Nevertheless, in an occasional patient abnormal fibrinolytic activity seems to be responsible for initiating or potentiating a bleeding tendency.

TABLE I Some Inhibitors of Plasmin and of Clot Lysis

Sub- strate	Substance Tested	Minimal Inhibitory Concentration (moles per L.)
	Cysteine	2 × 10 ⁻²
Casein*	Dimercaprol	3 × 10 ⁻⁴
	Lugol's solution	3 × 10 ⁻⁴ ‡
	Cysteine	5 × 10 ⁻³
	Dimercaprol	3 × 10 ⁻³
	Lugol's solution	4 × 10 ⁻² ‡
Fibrin†	Parachloromercuribenzoic acid	10-4
	Quinone	10-4
	Hydroquinone	5×10^{-4}
	Sodium azide	5 × 10-4
	Sodium ascorbate	3 × 10 ⁻²

* Inhibition of digestion of casein by chloroformactivated plasmin, using a turbidimetric technic.68

‡Concentration of elemental iodine.

In fourteen years of active searching, we have seen only one patient⁶² in whom fibrinolysis seemed to be the primary mechanism underlying abnormal bleeding, and two or three other patients in whom fibrinolysis seemed to contribute to bleeding initiated by some other mechanism. With few exceptions, 56,58 in every convincing case report in the literature, the patient was undergoing surgery or childbirth or had suffered severe hemorrhage from the gastrointestinal tract or the uterus. Severe and often uncontrollable bleeding occurs from the site of the operative wound, from the raw placental site, from the sites of venipunctures or parenteral injection, and rarely from the supposedly intact gastrointestinal tract. It is not surprising that the major sources of bleeding should be from injured tissues. It must be recalled that in patients with congenital afibrinogenemia months and even years may elapse without any signs of bleeding. In other words, even were plasmin to destroy all fibringen in the circulating plasma, this of itself would not be enough to produce bleeding. Bleeding in afibrinogenemia results only when the body's hemostatic mechanisms are challenged. Furthermore, in virtually every case in which fibrinolysis seems to be responsible for bleeding, careful studies show an appreciable concentration of fibrinogen within the circulating plasma. Presumably, bleeding occurs because the fibrin which forms at the site of an injury is lysed before other reparative measures can provide hemostasis. One wonders whether any plasmin formed within the blood stream is neutralized by the potent inhibitors present in the plasma. The plasminogen which is adsorbed to the fibrin clot forms plasmin which is in intimate content with its substrate, so that fibrinolysis results.

Treatment of Fibrinolytic Purpura: The treatment of fibrinolytic bleeding is difficult, and few patients have survived. The transfusion of blood and of fibrinogen may be helpful to correct blood loss and to increase the concentration of circulating fibrinogen. Heuson65 has suggested the use of soybean trypsin inhibitor, since this substance will neutralize the action of plasmin.66 However, soybean trypsin inhibitor is also an anticoagulant,67 and we should hesitate to use it in the care of these patients. In unpublished experiments we have found that the digestion of casein by plasmin was inhibited by cysteine, dimercaprol and Lugol's solution. Furthermore, the lysis of a fibrin clot was inhibited by these agents as well as by parachloromercuribenzoic acid, quinone, hydroquinone, sodium azide and sodium ascqrbate (Table 1). However, it seems doubtful that these agents could be used therapeutically.

COMMENTS

One conclusion may be drawn from the study of patients with fibrinolytic purpura in regard to the use of fibrinolytic agents to treat intravascular thrombi. The ideal therapeutic agent should be an activator which will be adsorbed to the fibrin clot so that plasmin will form at the site of the thrombus. Then, once lysis has occurred, the freed plasmin can readily be neutralized by the circulating inhibitors of plasmin before it can destroy the many other proteins which are its substrates.

Finally, we should like to return to the teleologic question posed at the start of this paper. It is in many ways a glib explanation to suggest that the role of plasmin is to lyse fibrin clots which may have formed at inadvertant times or places. An important property of plasmin is that it converts the first component of complement to an esterase. Unpublished studies of Hinz and of Lepow have demonstrated clearly the important part played by the esterase derived from complement in the immune mechanisms of the body. Perhaps when all is said and done

[†] Assayed by measuring the clot lysis time at 37 °C. at a mixture of 0.5 ml. oxalated plasma, 0.1 ml. test substance and 0.2 ml. 0.05 M calcium chloride solution.

we have been looking at the problem from the wrong point of view and it may be that we will find that for the true physiologic function of this enzyme we must look to its role in the immune defenses of the body.

SUMMARY

Spontaneous fibrinolytic activity, as tested in vitro, is more rapid when the plasma is obtained from a person undergoing stress. The mechanisms responsible for this rapid lysis and its physiologic meaning are obscure. The rapid lysis of blood drawn after exercise or childbirth is not accompanied by a fall in the concentration of fibrinogen in the plasma. Excessive fibinolysis is also noted in patients with chronic hepatic disease, but its relation to the hemorrhagic complications of such disorders is not known. Rarely is fibrinolysis so violent and rapid that it appears to be responsible for a hemorrhagic state. With few exceptions, in every convincing case report in the literature the patient was undergoing childbirth or surgery or had suffered severe hemorrhage. In virtually every instance, careful studies showed an appreciable concentration of fibrinogen in the circulating plasma. Studies of patients with fibrinolytic purpura support the contention that the ideal therapeutic agent for the solution of intravascular thrombi would be an activator which would be adsorbed to the fibrin clot, rather than an active proteolytic enzyme such as plasmin.

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Discussion of Paper by Drs. Ratnoff and Donaldson

DR. F. K. Beller (Stuttgart, Germany): I would like to ask Dr. Ratnoff how to interpret at the present time the role of fibrinolysis associated with obstetrical complications such as afibrinogenemia. According to the analysis of our cases, we have observed fibrinolysis most often following premature separation of the placenta (abruptio placentae). Since other authors are not able to confirm these findings, we consider these discrepancies to be due to variation in the time of venipuncture which is often carried out only once.

Certainly, fibrinolysis does occur more frequently in these cases. But what is the nature of its genesis? The following possibilities are usually discussed: (1) Autoextraction of fibrinokinase corresponding to that of thromboplastin. But until now neither any active fibrinokinase has been found to be present in the placenta nor has the existence of an (plasminogen) activator been proved. (2) In Germany we often refer to the reports of Hoff pointing out the possible relationship between shock and the development of a fibrinolytic activity passing through a vegetative intermediate phase. (3) Fibrinogen digestion may occur followed by an alteration of the ratios of ferment and substrate. (4) Afibrinogenemia may be initiated by a state of hypercoagulability resulting in a secondary fibrinolytic response. It is not certain whether fibrinolysis occurs here as a pathogenetic process or as a physiologic response of the organism to lyse preformed thrombi. In this case antifibrinolytic therapy might be contraindicated. Since all of these mechanisms are not very clear we have hitherto preferred the term "concomitant fibrinolysis."

DR. ROBERT L. CLARKE (New York, New York): I would like to comment on the question of the spontaneous disappearance of fibrinogen and the incoagulability of blood. In connection with sudden death, we recently performed a series of experiments in presumably unstressed, normal dogs and in animals that underwent stress. The animals were killed by exposure to high altitude, and blood samples were withdrawn from the venous pool. We confirmed the findings of others that shortly after death there is massive coagulation in animals that underwent stress whereas there is disappearance of fibrinogen but no coagulation in unstressed animals. When the unstressed animals were given heparin immediately prior to death fibrinogen disappearance was not marked, and after a relatively long period of time, we observed coagulation. On the other hand, when we

gave antifibrinolysin to unstressed dogs that were subsequently sacrificed, we again observed that the rate of fibrinogen disappearance was sharply decreased and that, over a long period of time, a clot was formed. It was apparent that both the coagulation process and the fibrinolytic process, be the latter by kinase or whatever mechanism, had to be present and functional in order that this disappearance of fibrinogen or the absence of coagula be observed in unstressed animals following sudden death. It appeared as though some kind of intermediate was formed from fibrinogen, which apparently never formed a coagulum but simply disappeared. When heparin was present to block the formation of this intermediate from fibrinogen or when antifibrinolysin was present to prevent its removal by fibrinolysin, the system could not progress. This substance is presumably soluble, is apparently capable of inhibiting coagulation and is, at the same time, hypersusceptible to fibrinolysin, although the exact mechanism is not known.

DR. MURRAY WEINER (New York, New York): Dr. Ratnoff's discussion of the importance of distinguishing fibrinolysis from retraction and problems concerning fibrinogen concentration in methods that are in common use is of great interest. Here, the "thrombelastogram" has advantages. For example, a reduced ability to retract or a reduced fibrinogen concentration will show itself as a lower maximum amplitude on the thrombelastograph, in contrast to lysis which will reach a given amplitude which then goes progressively down from the maximum as lysis develops and continues. On the other hand, it is true that having a low amplitude does not ensure the diagnosis of low fibrinogen level. This problem is intimately wound up with the problem presented by Dr. Fearnley concerning the importance of the nature of the clot in which lysis is being observed. The point about the difference between the calcium clot and the thrombin clot is readily demonstrable, but I think there are many more subtle variants that depend on the nature of the clots being dissolved, particularly if it is made of so-called purified fibrinogen or a variety of other preparations. In the problem discussed earlier concerning lipemic blood, for example, we have observed that, with our method, after an intravenous infusion of fatty material we did not note any spontaneous lysis but the clot which formed in some instances had a reduced firmness as determined by the maximum amplitude of the thrombelastograph. It might be expected that this clot, as compared to a normal clot, subjected to the same fibrinolytic mechanism, would show a difference in dissolution.

One very interesting point about Dr. Ratnoff's discussion was the relationship of hepatic disease to problems of fibrinolysis. One must seriously consider the possibility that patients with hepatic disease demonstrate lysis because of a deficiency of inhibitor. In most patients given nicotinic acid intravenously

fibrinolytic activity developed. Giving the same or much larger doses orally (which we know are absorbed because the patients flush) did not result in fibrinolytic activity except in patients who had undergone a portacaval shunt operation. This suggests that the nature of the first circulation of this fibrinolytic activator, through the liver vs. not through the liver, makes an important difference in whether or not fibrinolytic activity becomes demonstrable.

DR. RATNOFF (closing): I was asked what mechanisms are responsible for hypofibrinogenemia in premature separation of the placenta. The firmest data are those of Pritchard (PRITCHARD, J. A. and WRIGHT, M. R. Pathogenesis of hypofibrinogenemia in placental abruption. New England J. Med., 261: 218, 1959). Pritchard provided experimental verification of the hypothesis originally proposed by Dieckmann. He found that the amount of fibrin found in the retroplacental hematoma accounted for the great bulk of the fibrinogen which had disappeared from the circulating plasma. It is true that in some patients with premature separation of the placenta there may be some increased fibrinolytic activity, as measured in the test tube. Certainly, such patients might well have the increased fibrinolytic activity which is observed in other patients with

hemorrhage, shock, stress and exercise, all of which are elements in this disorder. Still, such increased fibrinolytic activity is probably not the fundamental defect responsible for hypofibrinogenemia in premature separation of the placenta. Only rarely does one find fibrinolytic activity of great magnitude in these patients.

We have been unable to demonstrate significantly rapid fibrinolysis in the blood of many patients under conditions which other investigators have associated with this state. I recently saw two patients, one with septic abortion and the other with pneumococcal sepsis, who had no detectable fibrinogen within their circulating system. We were unable to demonstrate any evidence of increased fibrinolytic activity in the plasma of either of these patients.

Dr. Weiner's comments about hepatic disease are interesting. It has been known since Goodpasture's study in 1914 that the increased rate of fibrinolysis in this disease cannot be correlated either with an increased concentration of what we now call plasminogen or a decreased amount of the plasminogen inhibitors in the circulating plasma. The increased rate of fibrinolysis can be correlated only with the rate at which the inhibitory activity of the plasma against plasmin deteriorates in the test tube.

Purification of Components of the Plasmin System*

DANIEL L. KLINE, PH.D. and JACOB B. FISHMAN, PH.D.

New Haven, Connecticut

THE MODERN phase of the development of methods for the purification of plasminogen was ushered in by Remmert and Cohen in 1949.1 Using isoelectric precipitation and kaolin as an adsorbing agent, these investigators obtained considerable concentration of activity from serum. However, the yields were very low and the reproducibility unsatisfactory. In 1950, Christensen and Smith² introduced the use of lyophilized Cohn fraction III as a starting material and strong mineral acid as an extraction agent. Unfortunately, this method gave extremely erratic yields and purities. A satisfactory procedure for the concentration of plasminogen from fraction III was worked out and published by our laboratory in 1953.3 This procedure provides the basis of more recent modifications. The usual purity obtained, 50 to 60 casein units/mg. N, was increased somewhat by a modification introduced in 1954.4 Further modification and the use of frozen liquid rather than lyophilized fraction III enabled Sgouris, Inman and McCall⁵ in 1959 to obtain preparations showing 92 units/mg. N.

A new major step forward has just been reported by Hagan, Ablondi and de Renzo.⁶ Starting with plasminogen purified by our earlier methods, they have obtained preparations after chromatography on carboxymethyl cellulose columns at an acid pH which have purities ranging from 140 to 200 casein units/mg. N depending on the material put on the column. Biochemical and biophysical studies⁷ indicate that their purest products are virtually homogeneous. This affords the possibility of an attack on the elucidation of the amino acid sequence of the molecule although the reported molecular weight, 83,800, is fairly high and introduces complicating but not insurmount-

able difficulties. Additional insight into the conversion of plasminogen to plasmin should also be possible with the use of this material. The ratio of plasminogen to proactivator activities of this highly purified, electrophoretically homogeneous material was the same as that of plasma. Alkjaersig⁸ has been able to purify plasminogen by the chromatography of euglobulin. This exciting development yielded a soluble product and may eliminate the use of fraction III, a highly variable source of plasminogen.

PLASMIN

A review of the literature fails to reveal any procedure for the isolation of plasmin prepared from purified plasminogen. A commercial fibrinolysin, actase, is stated to be such a preparation, but details of its purification have not been published and contamination with streptokinase-activator, the plasminogen activator produced by streptokinase (SK), has been reported.9 The complete conversion of plasminogen to plasmin has been accomplished with the use of glycerol, trypsin and urokinase. 10 The plasmin formed presumably had an activity approximately equal to that of the plasminogen used, 50 to 60 casein units/mg. N. However, isolation of the plasmin was not reported. Streptokinase has also been used for the conversion of plasminogen to plasmin, but the product isolated was a mixture of plasmin and the SK-activator. We11 have recently developed a procedure for the isolation of plasmin after the conversion of plasminogen in the presence of minimal amounts of SK. The plasminogen solution was activated with 25 units of SK/ mg. enzyme and was then dialyzed against water. Plasmin was precipitated at low ionic strength by the addition of NaCl solution at

^{*} From the Department of Physiology, Yale University School of Medicine, New Haven, Connecticut. This work was aided by grant NSF-G7496.

TABLE I Plasmin Purification

Plasminogen (casein units/mg. N)	Plasmin (casein units/mg. N	
8.4	74	
46	120	
52 (5)	95 (5)	
59 (2)	96 (2)	
65 (2)	108 (2)	
79 (2)	180 (2)	
81	191	

Note: Numbers in parentheses indicate the number of experiments.

pH 8.5. The product was water soluble and purer in terms of casein units/mg. N than the starting plasminogen. Table I shows the results of a series of preparations made from plasminogen of different specific activities. The purity of the final product was related to the plasminogen used.

Since SK was used as the activator, it was important to determine whether or not SKactivator was a contaminant of the plasmin isolated. The detection of trace quantities of activator in the presence of large amounts of plasmin is difficult since their activities produce similar end results. We first demonstrated that the addition of this isolated plasmin to plasminogen did not result in an increased hydrolysis of casein (Table 11). In addition, a new test for SK-activator activity was devised which depends on the finding that plasmin in the presence of soy bean inhibitor does not measurably attack casein, lysine methylester or dissolve a purified clot. SK-activator activity is not altered by the soy bean inhibitor. In the presence of increasing amounts of soy bean inhibitor, an activator preparation which contained both activator and plasmin showed de-

TABLE II

Absence of Activator Activity in Purified Plasma
Preparations

Experi- ment	Plasmin (casein units)	Plasmino- gen (casein units)	Total (casein units)	Plasmin + Plasmino- gen (casein units)	Difference (casein units)
1	0.80	0.59	1.39	1.45	0.06*
2	0.78	0.43	1.21	1.23	0.02*

^{*} Not significant.

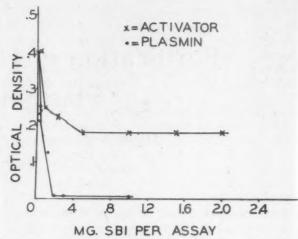


Fig. 1. Absence of lysine methylester activity by plasmin in the presence of soy bean inhibitor (SBI) and inhibition of plasmin but not of activator activity in a preparation containing activator and plasmin. Note the plateau of inhibition reached after suppression of plasmin was complete.

creasing esterolysis of lysine methylester until a plateau was reached. The further addition of soy bean inhibitor did not further diminish splitting of lysine methylester (Fig. 1). The addition of soy bean inhibitor to the plasmin preparation, however, resulted in a rapid loss of lysine methylester activity to a zero level, indicating that it was free of detectable SKactivator. Since clot lysing is far more sensitive than esterolytic tests, soy bean inhibitor was added to an activator plus plasmin and to a plasmin preparation; these were then tested on unheated bovine fibrin plates. Table III shows that the greatest lysis occurred with the activator plus plasmin. Soy bean inhibitor reduced this by eliminating the effect of the plasmin. The purified plasmin produced smaller areas of lysis and, after the addition of soy bean inhibitor, failed to lyse the fibrin at all. These tests, then, have all given a negative

TABLE III
Absence of Activator in Purified Plasmin

	Lysed Area ⁴ (mm ²)
Activator-plasmin mixture	1,373
Activator-plasmin + soy bean inhibitor	765
Purified plasmin	416
Purified plasmin + soy bean	
inhibitor	0

^{*} Unheated bovine fibrin plates; SK control, negative.

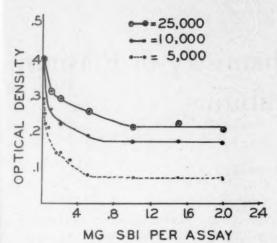


Fig. 2. Increased yields of activator as SK to plasminogen ratio was increased as measured by LME splitting in presence of soy bean inhibitor. Curves: 5,000, 10,000 and 25,000 SK units/mg. of plasminogen (8 casein units/mg.). Plasmin formation simultaneously decreased.

answer to the possibility that SK-activator is a contaminant of the new plasmin preparation.

STREPTOKINASE ACTIVATOR

The isolation of the plasminogen activator which is formed when SK is added to human plasminogen preparations has not as yet been accomplished. The only published method12 yields, after alcohol fractionation, a mixture of activator and plasmin from which it has not been possible to remove the plasmin. We subsequently found (reported also by Alkjaersig, Fletcher and Sherry¹³) that the production of activator increased with an increase in the SK to plasminogen ratio employed. product isolated by alcohol fractionation was likewise richer in activator as more SK was used. This increase was accompanied by a decrease in the plasmin yield (Fig. 2). With 25 units of SK/mg. of plasminogen, almost the entire activity was attributable to plasmin. As the ratio of SK to plasminogen was increased, the ratio of activator to plasmin formed and isolated also increased. With 25,000 units of SK/mg. of enzyme, we obtained a product which contained 47 per cent plasmin and 53 per cent activator activities. The activator is extremely labile. Even small changes in salt concentration or pH may destroy the

activity. The material, however, can be lyophilized. Both plasmin and activator can be precipitated from solution by prolonged dialysis against distilled water. We are now attempting to separate the plasmin from the activator by chromatography on cellulose columns.

SUMMARY

Methods for the purification and isolation of plasminogen, plasmin and the SK-plasminogen activator are reviewed. In addition, a new method is presented for the isolation of highly purified plasmin, and preliminary data are presented concerning attempts to isolate the SK-plasminogen activator in purer form than has hitherto been possible.

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Some Aspects of the Chemistry of Plasmin and Its Inhibitors*

PHILIP S. NORMAN, M.D. Baltimore, Maryland

PLASMIN is the active form of a naturally occurring serum protein, plasminogen. Plasminogen is acted upon by a number of activators; and the resulting plasmin is capable of digesting protein substrates, particularly fibrin. Plasmin, however, is also inhibited by other serum proteins called antiplasmins. Study of the interactions of plasminogen with activators, and of the resulting plasmin with antiplasmins and substrates allows inferences to be made about the physiologic activity of these proteins as well as the practicality of the use of several of them as therapeutic agents in thrombotic diseases.

Three naturally occurring serum proteins have been identified as components of the fibrin digesting system: (1) plasminogen, (2) alpha-1-antiplasmin, (3) alpha-2-antiplasmin. There may be one or more other components but they have not been well characterized and their existence is still problematical.

ACTIVATION OF PLASMINOGEN

Plasminogen, the inactive precursor, is a beta-globulin present in the blood of all persons and, when converted to plasmin, is an active proteolytic enzyme which has a remarkable capacity for digesting the fibrin matrix of clots. The greatest interest at present is in the safe and efficient use of the clot digesting property of plasmin to digest thrombi. Plasmin apparently digests fibrin and other proteins by hydrolysis of peptide bonds at arginine and lysine residues and may be shown to attack esters and amides of these two amino acids. These synthetic substrates are also competitive inhibitors of the proteolytic activity of plasmin.¹

The mechanism of conversion of plasminogen to plasmin seems to be the removal of a

moiety of the plasminogen, so uncovering an active center. This hypothesis is supported by physical studies that suggest that plasmin is a smaller molecule than plasminogen.² Furthermore, most known activators are hydrolytic enzymes which digest amide or ester linkages, and activation can be inhibited by the esters of arginine or lysine which are substrates for these activators.³

Action of Streptokinase: Although streptokinase is the most potent activator and has been thoroughly studied, the mechanism of its action is not completely understood and seems to be a special case; for it cannot be demonstrated to be a protease or esterase, even though activation of plasmin by streptokinase is inhibited by lysine or arginine esters. Streptokinase is also unique among activators in its species specificity, being virtually inactive with the plasminogen of any species but man. In conjunction, however, with the globulins of human serum, streptokinase becomes able to activate the plasminogen of any species.

Coactivator Property of Serum: These considerations have led to the hypothesis that streptokinase acts through some intermediary in human serum. It has been suggested that there is a coactivator in human serum which streptokinase converts to an activator, which in turn converts plasminogen to plasmin.4 Hypotheses such as this are valuable in that they suggest further experiments. If such a coactivator exists it should be separable from the other components of the plasmin system. When a number of different technics of protein separation have been tried, it has been found invariably that coactivator property is always to be found with plasminogen. Kline and Fishman⁵ reported that, in purification by acid extraction

^{*} From the Infectious Disease and Allergy Division of the Department of Medicine, Johns Hopkins University and Hospital. This work was supported by a grant from the National Heart Institute, U. S. Public Health Service and by a contract with U. S. Army Chemical Corps, Fort Detrick, Frederick, Maryland.

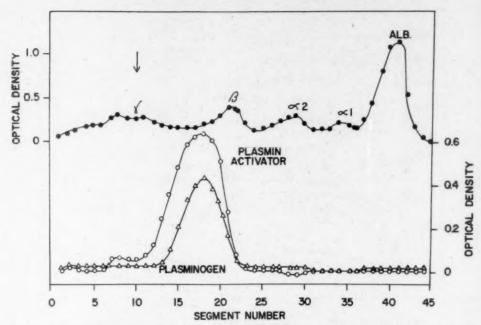


Fig. 1. Electrophoretic mobility in starch of the plasminogen and plasmin coactivator in human plasma. Upper line shows protein concentration as measured by optical density at 280 m μ . Lower lines show plasminogen and coactivator measured by casein digestion after streptokinase activation.

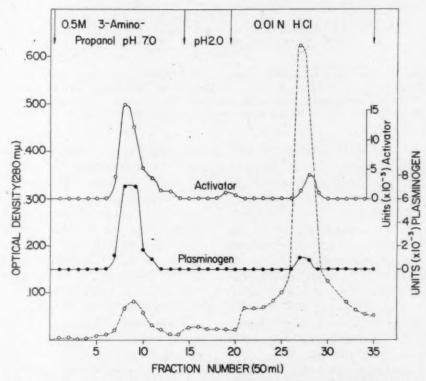


Fig. 2. Chromatography of purified plasminogen on diethylaminoethyl cellulose. The same methods of determination were used as in Figure 1.

and differential denaturation at alkaline pH, coactivator was always concentrated with plasminogen. Plasminogen and the coactivator property of human plasma have the same

electrophoretic mobility; for, as shown in Figure 1, they appear in the greatest amount in exactly the same eluates from zone electrophoresis in starch. Recently we have been

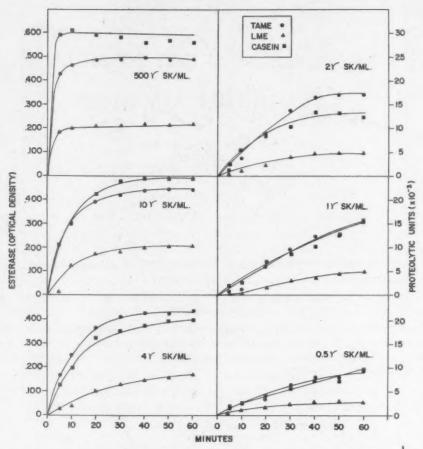


Fig. 3. Activation of proteolytic and esterolytic activity of purified plasminogen by streptokinase (Varidase®) at 25°c. Aliquots were withdrawn at the indicated times and the reaction stopped by precipitation of plasmin with 1 M NaCl at pH 2.0. (TAME = tosyl arginine methyl ester, LME = lysine methyl ester)

able to analyze the protein of a highly purified preparation of plasminogen made by the technic of Kline⁶ into three components by chromatography on cellulose. This experiment is illustrated in Figure 2, which shows the chromatogram obtained by stepwise elution of the protein from a cellulose column with 3-amino propanol and 0.01 N hydrochloric acid. As with other technics of separation, the coactivator property is found only in the proteins containing plasminogen. So far as I know, it has not been possible to prepare either plasminogen or plasmin that does not exhibit activator when streptokinase is added to it.

If the coactivator theory is correct, activation is a two-step enzymatic reaction which should show certain kinetic properties. It will be obvious that a certain amount of coactivator must first be generated before plasmin itself can begin to be formed; consequently, in an experiment wherein the appearance of plasmin produced by streptokinase is followed closely, the time

required for an enzymatic conversion of coactivator to activator will appear as a lag period. As shown in Figure 3, no such lag period can be demonstrated when the activation of plasmin by a range of concentrations of streptokinase from low to high is followed carefully, even though the rate of activation is made rather slow by lowering the temperature to 25°c. It is also demonstrated that the development of esterase activity closely parallels the development of proteolytic activity.

Streptokinase Plasmin Combination: The foregoing experiments fail to support the concept
of enzymatic conversion of coactivator to activator by streptokinase. There is, however,
some evidence of a loose combination between
plasmin and streptokinase. As shown in Figure
4, streptokinase is capable of a competitive
inhibition of the proteolytic activity of plasmin
so that when high concentrations of streptokinase are present plasmin may be more than
50 per cent inhibited. Streptokinase also

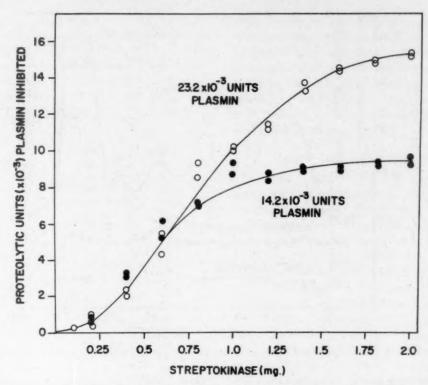
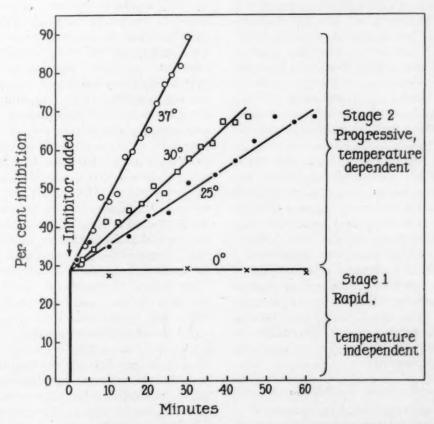


Fig. 4. Inhibition of the casein digestion of purified plasmin by streptokinase (Varidase).



 $F_{\rm IG}$. 5. Per cent inhibition of purified human plasmin by serum at different temperatures. A two-stage reaction is demonstrated.

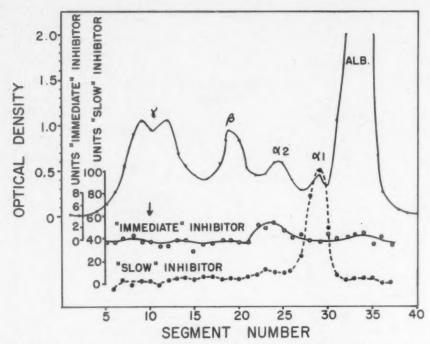


Fig. 6. Electrophoretic mobility in starch of the antiplasmins in human plasma.

inhibits the autodigestion of plasmin. Apparently the combination is reversible; because, upon exposure to pH 2, the streptokinase is denatured and plasmin activity completely restored. Furthermore, at the time of exposure to pH 2, the activator property is lost but may be restored by bringing the reaction back to pH 6.6 and adding fresh streptokinase. The process of destruction and regeneration of activator may be repeated many times without loss of the activity regenerated, an unlikely eventuality if there were an enzymatic conversion of a coactivator.

These facts suggest a different hypothesis about the action of streptokinase: namely, that it combines loosely with human plasmin and in so doing accelerates the known capacity of plasmin to activate plasminogen, although the combination has lowered capacity to digest other proteins. This hypothesis satisfactorily accounts for the lack of enzymatic activity of streptokinase alone even though esters of arginine and lysine are inhibitors of streptokinase activation. It also satisfactorily explains the kinetics of activation and the potentiality of plasmin preparations for many regenerations of the activator property.

INHIBITORS OF PLASMIN

Whatever the mechanism of activation of plasmin, once it is formed it becomes available to action of inhibitors in normal serum. It is

now possible to give some of the details of the inhibitory reactions. Observation of the rate of inhibition of purified plasmin upon addition of whole serum at various temperatures demonstrates a two-stage reaction (Fig. 5). Immediately, there is an inhibition quantitatively independent of the temperature; this is followed by a slower reaction which is highly dependent on temperature. Further study of the characteristics of each phase has shown that the first is too rapid to measure but is readily reversible by a protein substrate. There seems, therefore, to be a competitive inhibitor which, because of its competitive nature, not only modifies the action of plasmin but is also incapable of complete inhibition. The slower phase, which is dependent on temperature, appears to be due to a bimolecular reaction of an inhibitor with plasmin forming an inhibited plasmin that is not readily regenerated. After elapse of some time plasmin is completely inhibited. The ability of plasma to inactivate plasmin by this reaction is great, being some thirty times that of the quantity of potential plasmin to be found in normal blood.8

Alpha-Globulin Inhibitors (Antiplasmins): When human plasma was subjected to electrophoresis in a starch medium, and the fractions so isolated were tested for their ability to inhibit plasmin, it was shown that these two properties are found in different serum proteins (Fig. 6). The immediate inhibitor migrates with alpha-2-

Table 1 α1-Antiplasmin, α2-Antiplasmin, and Plasminogen in Plasma from Normal Humans

Case No.	Sex	α1-Antiplasmin (Units × 10 ⁻³ /ml.)	α2-Antiplasmin (% Inhibition*)	Plasminogen (Units × 10 ⁻³ /ml.)
1	М	800	54.5	31.4
2	M	990	60.4	26.2
3	M	760	54.0	37.9
4	M	960	58.0	28.8
5	M	660	49.5	24.4
6	F	900	58.0	20.0
7	M	700	54.5	31.0
8	F	950	57.2	21.0
9	M	775	52.0	30.3
10	M	940	54.8	24.2
11	M	785	55.5	30.5
12	\mathbf{M}	800	55.6	27.6
13	F	820	57.0	27.9
14	F	810	58.0	24.4
15	F	920	61.2	31.0
16	M	870	57.2	28.4
17	M	950	61.2	30.4
18	M	910	57.2	23.0

^{*} Calculated as the per cent inhibition obtained in a standard caseinolytic test.

globulins and the slow inhibitor migrates with alpha-1-globulins. For this reason it has been suggested that these two inhibitors with differing rates of reaction with plasmin be called alpha-1-antiplasmin and alpha-2-antiplasmin. Table 1 shows the amount of alpha-1-antiplasmin, alpha-2-antiplasmin and plasminogen measured in the plasma from a group of normal human subjects. In normal people the levels of these substances seem to vary within rather narrow limits and consistently there is twenty to thirty times as much inhibitor as plasminogen.9

The reactions of serum inhibitors with plasmin are profoundly affected by the presence of a protein substrate. The inhibition by alpha-2-antiplasmin is readily reversible by an increase in the concentration of a protein substrate, whereas the inhibition by alpha-1-antiplasmin, although not readily reversible, does not progress further in the presence of a substrate. These facts explain why a blood clot bathed in plasma can be digested even though potent inhibitors are present. Fibrin, as a typical substrate, is more avid for the active site of plasmin than antiplasmin. Thus, the plasmin-fibrin interaction that results in fibrinolysis takes precedence over the combina-

tion between plasmin and inhibitor. Only when there is no more fibrin present do the inhibitors combine with plasmin. Fibrinogen, although a substrate for plasmin in purified systems, seems to be less avid for plasmin than inhibitors, and Celander and Guest¹⁰ have shown that plasmin may be activated in whole plasma without loss of fibrinogen.

CHOICE OF THERAPEUTIC AGENTS

Plasmin: How may the knowledge of the properties of the plasmin system be used in choosing agents for treatment of blood vessels obstructed by clots? If plasmin is to be used, it must be administered at some site distant from the clot (intravenously, probably) and be carried in an active form by the circulation to the clot where it is to perform its fibrinolytic function. It will be evident, from the reactions described in this article, that a plasmin molecule, when injected, must run a gauntlet of many inhibitor molecules before it reaches the protection of the clot where it can attach to the fibrin substrate. Despite the rapid loss of plasmin activity which must occur in the blood, it may be possible for some plasmin molecules to arrive at the clot and still be active. The combination with one of the inhibitors is readily reversible and does not block the digestion of fibrins. The other inhibitor (alpha-1-antiplasmin) is less readily reversible; however, its reaction with plasmin takes time so that a portion of the injected plasmin may exist uninhibited in the circulation long enough to be carried to the clot. Ambrus, Back and Ambrus11 have recently obtained evidence that fibrin can actually reverse, in part, the combination of plasmin with the slow inhibitor. This is an important observation, if it is confirmed, because it means that some plasmin, even though in a rather firm combination with an inhibitor, can be freed for activity at the site of a clot. Whether this reversal is quantitatively important enough to be useful therapeutically has not been determined. In any event, it seems likely that much of any plasmin that is injected will be wasted because it will be inhibited. Whether plasmin without accompanying activators can be administered safely in amounts which will induce adequate lysis of thrombi in human disease still remains to be determined.

It seems unlikely that intravenously administered plasmin will induce afibrinogenemia, because the normal inhibitors are pro-

tective to fibrinogen. Whether other serum proteins, such as complement or clotting factors, are similarly protected is not known. Afibrinogenemia due to overdosage of plasmin could occur if enough plasmin were given to overcome the normal inhibitory power of the blood, but the high content of inhibitors in the blood provides a considerable margin of safety.

Plasmin Activators: A direct activator of plasmin, such as urokinase or fibrinokinase, would be subject to a somewhat different set of reactions when injected. Two different mechanisms are possible for the induction of thrombolysis by an activator: it might, in the circulation, activate plasmin which then would have to be carried to the clot and at the same time be subject to the inhibitory reactions just discussed; or the activator might be carried to the clot where it would activate the plasminogen in the clot. The latter seems to be a more efficient mechanism, because activators are not apparently subject to inhibitors in the blood and would be carried to the clot unchanged. Furthermore, the danger of hyperplasminemia due to overdosage is minimized, because the degree of plasminemia that would be achieved is limited by the blood content of plasminogen; it has already been shown that this is much less than the content of inhibitors.

Streptokinase: Most of the remarks made about direct activators also apply to the use of streptokinase, which is an indirect activator. The effectiveness of streptokinase probably depends on the availability of some blood protein, be it plasmin or coactivator, to activate plasminogen. A deficiency of this substance occurs in the blood or in clots only after infusion of large amounts of streptokinase.12 Streptokinase does have the disadvantage that it is an antigenic protein from the streptococcus and human beings usually have an antibody to it. Until the antibody is used up, it neutralizes injected streptokinase and so increases the dose needed for thrombolytic effects. The rise in serum antibody after an injection of streptokinase may preclude its further use for many months.18

A mixture of streptokinase and plasmin may be used in order not to depend on the body's supply of the substance needed for formation of activator. The addition of plasmin to streptokinase seems unnecessary in most situations because no naturally occurring deficiencies of plasminogen have ever been recognized and extremely large doses of streptokinase have been needed to deplete the blood plasminogen.12

Conclusions

On the basis of the current knowledge of the interactions of the components of the plasmin system it appears that a direct enzymatic activator of plasminogen would be the most useful thrombolytic agent while an indirect activator would be almost as efficient. The possibility exists that plasmin alone would be capable of therapeutic lysis of thrombi, but the intravenous injection of plasmin into a large pool of inhibitors does seem to be a wasteful means of producing thrombolysis.

These conclusions represent a logical extension of rather precise data on in vitro reactions and are, therefore, at best, only hypotheses which require experimental testing in vivo. A comparison of various thrombolytic agents which have been prepared free of contaminants causing side reactions is urgently needed in both animals with experimental thromboses and human subjects with thrombotic diseases. When making such comparisons, the importance of untreated control subjects is obvious, because the natural evolution of most untreated thromboses is toward at least partial improvement.

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DISCUSSION OF PAPERS BY DRS. KLINE AND FISHMAN; AND NORMAN

DR. FLETCHER TAYLOR (Oakland, California): Using the method described by Dr. Norman for study of inhibition, we have observed enhanced caseinolysis beyond that of the enzyme control in thirty cases of prostatic carcinoma and in ten normal subjects following exercise.

To test whether or not these enhanced caseinolysis levels were associated with rapid clot lysis times, both casein inhibitor and clot lysis (Fearnley) studies were performed as separate daily experiments on normal-prostatic carcinoma pairs. Thirteen pairs were studied. Faster clot lysis times were correlated with enhanced caseinolysis rates in ten of the thirteen pairs. "P" value was 0.008.

Enzymatic and fractionation studies are now being performed, using the casein substrate, in an effort to further characterize these naturally occurring activator, inhibitor substances; and to further elucidate their interrelationships with the partially purified enzyme. These studies include dialysis, ether extraction, pH, temperature and plasminplasma dilution studies. To date the evidence suggests that a single unit or molecular complex (e.g., inhibitor-enzyme) interacts with a second heat stable factor in the activation-inhibition balance of the enzyme system. Whether or not this factor is derived from the partially purified enzyme is important; also, those factors which favor the enhanced activity of the enzyme in certain diseases and in exercised normal subjects are important and merit further investigation.

DR. SAUL I. COHEN (West Roxbury, Massachusetts): Fundamentally, inhibition of plasmin depends on the enzyme's most important characteristic, namely its specificity. For a substance to be an inhibitor it must fulfill the substrate specificity requirements of the enzyme to a particular degree. This is borne out in our work which has shown that plasmin will hydrolyze peptide bonds in fibrin contributed by arginine and lysine, followed by release of free arginine and lysine, and by the fact that the amino acids lysine, ornithine and E-amino caproic

acid will inhibit plasmin. These observations do not preclude the probability that compounds far removed in structure from lysine will inhibit plasmin-fibrin interaction. I say this because a great deal of information indicates that interaction between an enzyme and a substrate is more than simple bonding at one particular site. Rather there are several links involved in the complex interaction between the reactive groups on the enzyme and substrate. The foregoing, it seems to me, would explain the alpha-2-globulin inhibitor described by Dr. Norman and suggests that the ideal natural inhibitor resembles fibrin more than it does lysine.

DR. JOHN MARKUS (Roswell Park, New Jersey): I quite agree with Dr. Norman's presentation of the mechanism of action of streptokinase. I think that it is a complex that is formed. In an experiment similar to that reported by Dr. Norman, caseinolytic and esterolytic activity was measured immediately after the addition of streptokinase; by using very short digestion times (five minutes) for both assays, we found that the caseinolytic and the fibrinolytic activities developed almost instantaneously, that is, in less than one minute, to their fuller extent whereas the development of TAME activity was considerably slower and reached its full extent only in about eight to ten minutes. We suggested, in a paper written in collaboration with Dr. Clara Ambrus (J. Biol. Chem., June 1960), that the first component with the early activity against fibrin and casein is probably identical with activator itself and is the result of the action of an enzyme consisting of a complex: plasminogen-streptokinase.

DR. BERNARD J. HAVERBACK (Los Angeles, California): Dr. Norman's work with serum plasmin inhibitors has paralleled our work with serum trypsin inhibitors. Serum contains very large amounts of substances which inhibit trypsin. One milliliter of normal serum has the inhibitory capacity to effectively neutralize slightly more than 1 mg. of crystalline trypsin. The serum trypsin inhibitors can be separated by electrophoresis, and they travel with the alpha-1- and alpha-2-globulin fractions. The major portion of the serum trypsin inhibitor travels with the alpha-1-globulin fraction while approximately 10 per cent travels with the alpha-2-globulin fraction. It should be pointed out that both serum trypsin inhibitor fractions combine rapidly with trypsin in contrast to the slow inhibiting action of the alpha-1globulin fraction with plasmin as shown by Dr. Norman. The question arises whether serum trypsin inhibitors can inhibit plasmin. My colleague, Dr. Hallie Bundy, has isolated the alpha-2-globulin trypsin inhibitors in a partially pure form on a diethylaminoethyl cellulose column. We have established that this substance inhibits plasmin as well as trypsin, but to a lesser extent. Current studies in our laboratory have been concerned with the levels of the alpha-1- and alpha-2-globulin trypsin inhibitors in acute pancreatitis. Our results indicate that in

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this disease there is a marked rise in the alpha-1-globulin trypsin inhibitor and a virtual disappearance of the alpha-2-globulin trypsin inhibitor. It would appear that the increase in the alpha-1-globulin trypsin inhibitor is not specific for pancreatitis as it increases in other diseases as well. However, as the alpha-2-globulin trypsin inhibitor markedly decreases in severe acute pancreatitis, the ratio of the alpha-1-to the alpha-2-globulin inhibitors markedly rises. It is likely that the change in this ratio will be helpful in the diagnosis of acute pancreatitis.

DR. E. C. DE RENZO (Pearl River, New York): Dr. Kline alluded to our paper which will appear in the Journal of Biological Chemistry and I would like to

summarize some of the data briefly.

A chromatogram of Kline type plasminogen, adsorbed on carboxymethylcellulose at low pH and eluted by simply lowering the pH by the addition of dilute hydrochloric acid, shows that the fast moving component is the major type contaminant and is devoid of activity. The slower moving component contains the plasminogen. The assay we employ is a true proactivator assay; that is, the system contains excess SK, excess bovine plasminogen and varying amounts of plasminogen fraction, to which, of course, we obtain a linear response in terms of lysine methylesterase activity.

The main plasminogen peak can be sharpened up a little more by chromatographing under the same conditions. From the curve of the units of proactivator activity per unit of protein it can be seen that there is a maximum reached which indicates there is some heterogeneity in the main peak.

In our experiments in which an increasing pH gradient was employed, we have achieved about a

six hundredfold increase in purity.

In addition to being assayed for proactivator, column purified preparations were assayed for plasminogen in terms of fibrinolytic, caseinolytic or tosyl-argininemethylesterase activities, all after activation with streptokinase. The point of interest is the maintenance of the constancy of ratios of these four activities (three activities relative to proactivator) over a six hundredfold purification from which we can fairly well conclude that if there are two molecularly distinct species, then they must be intimately associated. I should also like to mention that physicochemical studies indicate a high degree of

homogeneity by all criteria. Davies and Englert have made these studies in our laboratories (Journal

of Biological Chemistry, April 1960).

DR. PHILIP S. NORMAN (Baltimore, Maryland): Dr. Taylor's remark about the lack of inhibition in patients with prostatic carcinoma is of interest. We have looked for a deficiency of inhibition in other patients who were supposed to have fibrinolytic disease and were not able to find it. These were patients who had had fetal death in utero; we have not examined patients with prostatic carcinoma. Dr. Haverback's remarks about the nature of alpha-1- and alpha-2-inhibitors indicating that they have the same electrophoretic mobility whether they inhibit trypsin or plasmin suggests something which I have long suspected, that these inhibitors may be active against a number of different enzymes. In conjunction with Dr. Levy and Dr. Wagner we have found that inhibitors of the hemagglutinating activity of the influenza virus also migrate as alpha-1- and alpha-2globulins. Furthermore, antithrombin activity may be demonstrated to migrate with alpha-globulin. So it is quite possible, although we have not been able to prove it, that the inhibitory substances may be active against a number of different enzymes.

Dr. Markus' experiments, which find proteolytic and fibrinolytic activity early in activation by streptokinase, are well known to me. He does his experiments one way and I do mine another. Criticisms may be made of either technic and I am not able to decide which objections are most valid. Perhaps Dr. Markus and I can get together and do something to settle this. Chromatography of plasminogen on cellulose has been investigated in three different laboratories, Dr. Sherry's, Dr. de Renzo's and my own. Although entirely different technics have been used each of us is unable to separate plasminogen

from proactivator.

DR. DANIEL L. KLINE (New Haven, Connecticut): I would like to make it clear that I firmly believe in the existence of a proactivator in the streptokinase reaction. I believe that the molecule is also the plasminogen molecule, but proactivator activity is definitely established and, if it comes from one molecule or two, we must still find active centers or two active areas in order for the two separate reactions to occur.

DR. NORMAN: I suggest the hypothesis that streptokinase is an accelerator of self-activation by plasmin.

The Effect of Cysteine and Mercaptoethanol on Plasmin*

GERDA MOOTSE, PH.D. and EUGENE E. CLIFFTON, M.D.

New York, New York

THE EFFECT of sulfhydryl compounds on proteolytic enzymes has been extensively studied. It has been reported¹ that the sulfhydryl group has an inactivating action on trypsin and chymotrypsin. It has also been shown that inactivation of trypsin in the presence of 8 M urea occurs at lower concentration of reducing substances and much faster. The idea has been expressed that the inactivation is connected with reduction of the disulfide bond.²

In 1959 Orekhovich et al.³ reported that 0.05 M L-cysteine did not inhibit the hydrolysis of serum albumin by trypsin. Whether the effect of thiols is due only to inactivation of the enzyme or also to inactivation of the whole course of digestion should be reinvestigated. Plasmin, the proteolytic enzyme, has many similarities to trypsin.⁴ .⁵

Complete destruction of fibrinolytic activity in 1 per cent solution of cysteine and of glutathione has been reported by Guest et al.⁶ Cysteine has also been shown to be a strong inhibitor of the streptokinase-activated fibrinolytic activity of tissue extracts.⁷ White et al.⁸ demonstrated inhibition of bovine plasmin by thioglycolate on digestion of corticotropin-A.

In the experiments to be described, attempts have been made to study the *in vitro* effect of cysteine and mercaptoethanol on the case-inolytic and fibrinolytic system of plasmin.

MATERIAL AND METHODS

Material: The following substances were used: Plasminogen was prepared from lyophilized fraction III (1955) by a modification of the methods of Kline⁹ and Mootse. ¹⁰ The material used in these experiments had a specific activity of 75 to 95 casein units per mg. nitrogen. The purified proenzyme was stored frozen.

Glycerol-activated plasmin was prepared by the method of Alkjaersig et al.¹¹

Bovine fibrinolysin solution was freshly prepared before use and kept on ice during the experiment.

Thrombolysin solution was freshly prepared before use and kept on ice during the experiment.

Streptokinase in stock solution was stored for about two weeks in the refrigerator.

Casein in stock solution was prepared from devitaminized casein, purified by the method of Norman¹² and stored frozen in a 4 per cent solution of 0.1 M phosphate buffer, pH 7.4.

L-Cysteine (free base) was freshly prepared every day. 2-Mercaptoethanol.

Urea.

Fibrinogen was purified by the method of Laki¹³ and freshly prepared every day.

Human fibrinogen.

Thrombin was freshly prepared every day.

Methods: Proteolytic activity was determined by a modification of the method of Remmert and Cohen. To 0.2 ml. portions of an appropriate concentration of plasminogen 0.5 ml. of the inhibitor solution was added. The mixtures were incubated at several different temperatures for varying times. Then, 0.2 ml. of streptokinase (SK) and 0.5 ml. of the same inhibitor were added and allowed to act at 37°c. for three minutes. After activation 1.1 ml. of 0.1 M phosphate buffer of pH 7.4 and 2.5 ml. casein were added and an aliquot (2 ml.) was withdrawn and blown into an equal volume of 10 per cent trichloracetic acid. Incubation of the remaining sample was continued at 37°c. for one hour when a second 2 ml. aliquot was withdrawn and treated in the same manner.

The absorption by duplicate filtrate was read in a Beckman DU spectrophotometer at 280 m μ . against a blank of water. Absorbancy readings were converted into γ of acid soluble tyrosine by reference to the standard tyrosine curve. The increase from the zero- to sixty-minute aliquots was taken as a measure of the proteolytic activity.

For inhibition studies of active plasmin 0.2 ml.

^{*} From the Clotting Mechanism Section of the Division of Experimental Surgery and Physiology, Sloan-Kettering Institute, and the Department of Surgery, Cornell University Medical College, New York, New York. This work was supported by Grant 2867 from the National Heart Institute, National Institutes of Health, and by institutional funds.

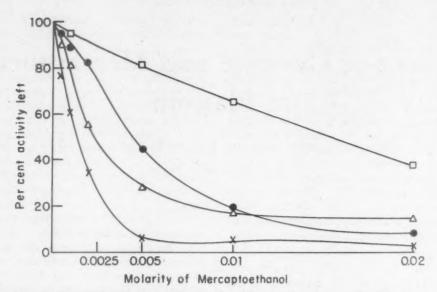


Fig. 1. The inhibition produced by different molarities of mercaptoethanol on the caseinolytic activity of plasmin having the activity of 75 to 95 casein units per mg. nitrogen. Activities expressed as the per cent activity left after one hour digestion. —— = plasminogen activated in the presence of substrate by 500 units of SK at 37°c. for three minutes. Activated enzyme incubated with inhibitor twenty minutes at 37°c. —— = twenty minutes' incubation in ice. $\Delta - \Delta$ = glycerol activated plasmin activity after incubation for twenty minutes at 37°c. X—X = plasminogen incubated with inhibitor for twenty minutes at 37°c., then activated with 500 units SK in the presence of a substrate.

plasminogen was added to 0.2 ml. SK. After three minutes' activation at room temperature of 37°c., 1 ml. of the inhibitor was added and continued as previously described.

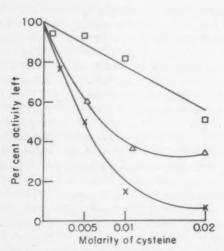


Fig. 2. The inhibition produced by different molarities of cysteine on the caseinolytic activity of plasmin having the activity of 75 to 95 casein units per mg. nitrogen. Activities expressed as the per cent activity left after one hour digestion. $\Box - \Box = \text{twenty minutes' incubation}$ in ice. $\Delta - \Delta = \text{glycerol activated plasmin activity}$ after incubation for twenty minutes at 37°c. $\times - \times = \text{plasminogen incubated}$ with inhibitor for twenty minutes at 37°c., then activated with 500 units SK in the presence of a substrate.

In the fibrinolytic method, an appropriate amount (0.1 ml.) of plasminogen was incubated with 0.2 ml. of inhibitor in veronal buffer before or after the addition of 0.1 ml. SK (the units are specified in the various tests). Next, 0.5 ml. fibrinogen (0.4 per cent in veronal buffer) and 0.1 ml. thrombin (1 unit) were blown into the tube, thoroughly mixed and placed in a water bath at 37°c. The time of clot lysis was recorded.

The biuret technic was that of Rosenthal and Cundiff,15 modified for plasma proteins according to Mootse.10

RESULTS

Effect of Mercaptoethanol and Cysteine on Case-inolytic Activity: Figures 1 and 2 illustrate the per cent proteolytic activity after twenty minutes of incubation with mercaptoethanol or cysteine (respectively). The results are qualitatively similar, but mercaptoethanol gave a more marked effect. It is interesting to note that the incubation at 0°c. with both of the inhibitors gave a linear relation.

Since it was noted that without incubation with the inhibitor (in 0.01 M to 0.02 M final concentration) there was always an immediate loss of caseinolytic activity during the one-hour digestion period, an effort was made to provide some explanation for it. Figure 3 shows two

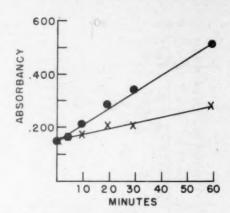


Fig. 3. Proteolytic activity (absorbency) developing during sixty minutes of digestion. •—• = to 0.2 ml. of plasminogen was added SK (500 units), then 1 ml. of 0.1 M cysteine and immediately followed by casein. X—X = to 0.2 ml. plasminogen was added 1 ml. of 0.1 M cysteine, immediately followed by SK and incubated for three minutes, then continued as mentioned.

activity curves developing in the presence of 0.02 M cysteine (final concentration) during a sixty-minute period. Activated plasmin ($\bullet - \bullet$) followed by cysteine gave about 40 per cent higher activity than the curve where SK was added after the cysteine (X—X).

Zero time incubation with mercaptoethanol (0.02 M final concentration) gave only about 30 per cent of the total activity. Figures for 0.01 M cysteine and mercaptoethanol were 97 and 62, respectively. Incubation of plasmin with the inhibitor before the addition of the bulk of the substrate gave additional loss of activity which was dependent on the time and temperature of the incubation.

Figure 4 shows the effects of time and temperature on the incubation.

In the second series of experiments the activity was recorded as the function of time of plasminogen incubation with cysteine. There was practically no change in activity by increasing the incubation from zero time to twenty minutes at 0°c. or at room temperature. Incubation at 37°c. gave some loss of proteolytic activity.

In attempts to localize the inhibitory activity of thiols at one or more of the steps in the whole system, the conditions as well as the concentration of the inhibitor (on incubation with plasminogen or plasmin and also in the final tube) were strictly controlled. The recoveries of the activities in both of the experiments were equal.

Data showing the inhibitory effect on glyc-

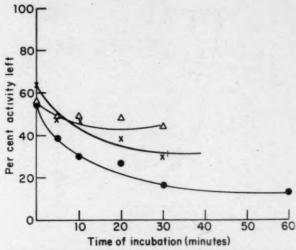


Fig. 4. The effect of various incubation times upon the inhibition of activated plasmin (500 units SK) by 0.02 M cysteine. X—X-plasminogen was activated in the presence of 0.3 ml. substrate for three minutes at 37 °c. The activated enzyme was incubated at room temperature for various times. $\Delta - \Delta =$ plasminogen activation at 37 °c. for three minutes without substrate, then incubated in ice. $\bullet - \bullet =$ no activation time, plasminogen and SK were followed by cysteine and incubation carried out at room temperature. All buffer controls showed only 2 per cent activity loss.

erol activated plasmin (Fig. 5) and bovine fibrinolysin (Fig. 6) are similar. Thrombolysin was the only preparation which, for some unknown reason, showed the same degree of in-

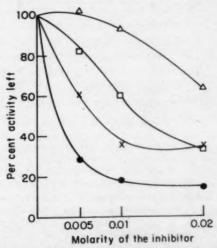


Fig. 5. The inhibition produced by different molarities of cysteine and mercaptoethanol on the caseinolytic activity of glycerol activated plasmin with and without incubation with the inhibitor. $\Delta - \Delta = \text{cysteine mixed}$ with the substrate and then added to the enzyme. $\Box - \Box = \text{mercaptoethanol mixed}$ with the substrate and then added to the enzyme. $\times - \times = \text{cysteine}$ incubated with plasmin for twenty minutes at 37 °c., then the substrate was added. $\bullet - \bullet = \text{mercaptoethanol}$ incubated with plasmin for twenty minutes at 37 °c., then the substrate was added.

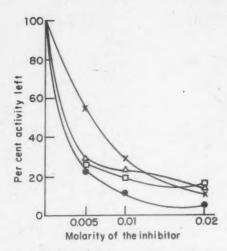


Fig. 6. The inhibition of proteolytic activity of bovine fibrinolysin or human thrombolysin when incubated twenty minutes at 37 °C. with cysteine or mercaptoethanol. $\times - \times =$ effect of cysteine on fibrinolysin. $\blacksquare - \blacksquare =$ effect of mercaptoethanol on fibrinolysin. $\square - \square =$ effect of cysteine on thrombolysin. $\Delta - \Delta =$ effect of mercaptoethanol on thrombolysin.

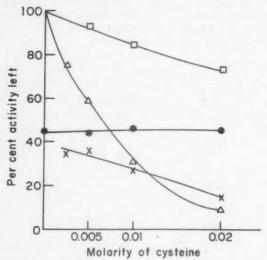


Fig. 7. Caseinolytic activity left after using a constant concentration of human plasma or euglobulin with varying molarities of cysteine. One milliliter of plasma was activated with SK in the presence (\times) or in the absence (\bullet) of 0.3 ml. of substrate at 37 °c. for three minutes. Cysteine was then added and incubated for twenty minutes at 37 °c. $\Box - \Box =$ activation in the presence of substrate, incubation with cysteine in ice. $\Delta - \Delta =$ 1 ml. euglobulin activated at 37 °c. for three minutes, then incubation for twenty minutes with cysteine.

hibition with cysteine and mercaptoethanol (Fig. 6).

Figure 7 illustrates the inhibitory effect of cysteine on plasma and on plasma euglobulin. There exists a linear relation on casein hydrolysis by plasma. It is noteworthy that without the protective action of substrate on activa-

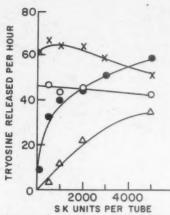


Fig. 8. Caseinolytic activity (acid-soluble tyrosine) as a function of SK units (per digestion mixture). $\times - \times =$ buffer control. $\bigcirc - \bigcirc =$ plasminogen incubated with cysteine (0.005 M final concentration) for twenty minutes at room temperature, then activated with various concentrations of SK at 37 °C. for three minutes. $\triangle - \triangle =$ cysteine in 1 M urea. $\blacksquare - \blacksquare =$ plasminogen activated at room temperature for three minutes, then incubated with cysteine in urea.

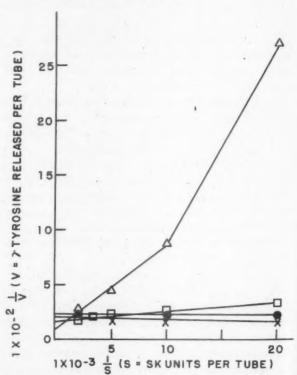


Fig. 9. The same data as in Figure 8 but plotted as suggested by Lineweaver and Burk. 17 $\times - \times =$ buffer control. $\Delta - \Delta =$ cysteine in urea, incubation before activation. $\Box - \Box =$ cysteine in urea, incubation after activation. $\bullet - \bullet =$ cysteine incubation before activation.

tion the cysteine sample in twenty mintues, incubation showed higher activity than the buffer control. The inhibition by cysteine seems to be more complex than anticipated and

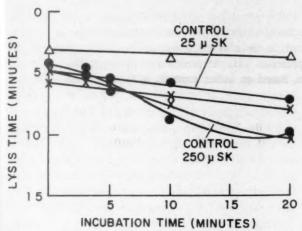


Fig. 10. The effect of time of incubation of activated plasma with cysteine (0.01 M final concentration). One-tenth milliliter human plasma was activated with 25 units SK or 250 units SK and incubated with cysteine at room temperature for various times. Then human fibrinogen (0.2 per cent final concentration) was added and clotted with 1 unit thrombin. $\bullet - \bullet = \text{controls}$. $\times - \times = \text{samples}$ with cysteine. $\Delta - \Delta = \text{plasma}$ incubated with buffer, then SK added. The scale is inverted so that high activities appear at the top of the figure.

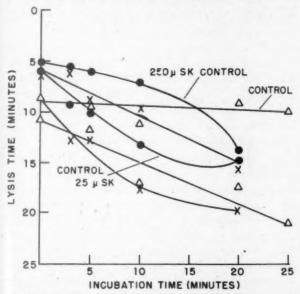


Fig. 11. This illustrated an experiment similar to that shown in Figure 10 with the exception that bovine fibrinogen (92 per cent clottable) was used. $\bullet - \bullet =$ controls. $\times - \times =$ samples with 0.01 M cysteine. $\Delta - \Delta = 150 \gamma$ plasminogen incubated with cysteine, next activated with 250 units SK control and sample.

this suggests that an investigation in greater detail should be undertaken.

The complexity was more apparent when cysteine was used in urea. In 1957 Norman¹⁶ reported the inhibitory action of urea on plasmin. But as he noted and as is confirmed in the present studies, casein was rendered somewhat more digestible by 1 M urea. To avoid an inhibitory

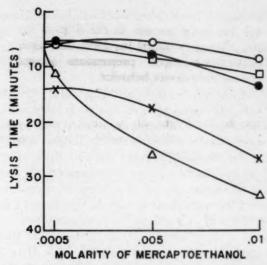


Fig. 12. The effect of concentration of mercaptoethanol on the hydrolysis of clot. Seventy-five gamma plasminogen was incubated with human or bovine (Armour) fibrinogen and mercaptoethanol at 37 °c. for twenty minutes. Next, 25 units SK or 250 units SK were added and clotted with thrombin. • • human fibrinogen, 25 units SK (hydrolysis of buffer controls was five hours, five minutes). $\Delta - \Delta = 250$ units SK (control eight hours, forty-four minutes). $\bigcirc - \bigcirc =$ bovine fibrinogen, 25 units SK (control five hours, forty-nine minutes). $\square - \square = 250$ units SK (control five hours, twenty-five minutes). $\times - \times =$ human fibrinogen (without plasminogen) 25 units SK (control twelve hours, forty-seven minutes). The scale is inverted so that high activities appear at the top of the figure.

action of urea, cysteine inhibition was carried out in 1 M urea. The inhibition of proteolytic activity of plasmin by cysteine was more pronounced in 1 M urea solution.

Figure 8 presents the caseinolytic activity as the function of the SK units per digestion mixture. High SK concentration (5,000 units SK) which were inhibitory for buffer controls, increased the caseinolytic activity in the presence of 0.005 M cysteine in 1 M urea above the control. Figure 9 gives the same data plotted as suggested by Lineweaver and Burk.¹⁷

Effect of Mercaptoethanol and Cysteine on Fibrinolysis: Investigation of fibrinolysis was much more complicated. Figures 10 and 11 present the hydrolysis of clots formed in the presence of active human plasma. Clots formed with human fibrinogen (Fig. 10) and 250 units SK increase their stability during the incubation with cysteine as well as with buffer. Plasma activated by 25 units SK was inhibited by 0.01 M cysteine.

Many factors and various experimental approaches were considered to analyze the hydrolysis of clots by purified plasminogen. Plasminogen incubation with inhibitors almost always gave an increase in the time of hydrolysis, linearly related to incubation time. Different plasminogen preparations showed

widely variable activity behavior.

Plasminogen incubation with inhibitor and human fibrinogen showed the protective action of fibrinogen for fibrinolytic activity (dependent on fibrinogen concentration). Bovine fibrinogen in high concentration showed little effect, but in low concentration it decreased the lysis time. Examination of the effect of mercaptoethanol concentration was made by following the hydrolysis in relation to mercaptoethanol mo-The results of this study are sumlarities. marized by the data in Figure 12. Human fibrinogen alone incubated with mercaptoethanol and activated by 25 units SK showed more inhibition than bovine fibrinogen incubated with mercaptoethanol. With 250 units SK bovine clots had a linear relation. The greatest decrease in activity was shown by human clots with 250 units SK. However, three minutes' activation with SK prior to clot formation re-established the fibrinolytic activity. Decreasing plasminogen concentration tenfold and using 25 units SK per tube, the lysis time of bovine clots was increased from twenty minutes to over twenty-four hours (0.02 M mercaptoethanol). Bovine clots (Fig. 11) formed in the presence of activated plasma are not so dependent on SK units as are the human clots. Twenty-five units as well as 250 units show inhibition with 0.01 M cysteine.

A few experiments were carried out with bovine fibrinolysin. Incubation with mercaptoethanol (0.01 M final concentration) for twenty minutes decreased activity markedly (clot lysis time over twenty-four hours).

Preformed clots with cysteine (0.01 M) immersed into plasminogen (375 γ activated by 250 units SK) showed a decrease of hydrolysis time (increased activity). The same effect was obtained when preformed clots without cysteine were immersed into plasminogen, cysteine (the same concentration) and 250 units SK solution.

Spontaneously fibrinolytic plasma euglobulin (lysis time one hour, forty minutes) from a patient with cirrhosis was inhibited by 0.01 M cysteine (lysis time greater than seven hours) and by 0.005 M mercaptoethanol (about five hours).

COMMENTS

These experiments demonstrated that cysteine and mercaptoethanol inhibit caseinolytic ac-

tivity of plasmin. The reducing agents in concentrations which do not affect the activity of plasmin in the absence of urea are inhibitory in its presence. In the presence of optimum SK units, based on buffer controls, with conditions strictly controlled, the same per cent of activity which will be activated by SK in a plasminogencysteine incubation mixture will be inhibited by the same SK units in the activated plasmin incubation with cysteine for the same incubation period. These results suggest an SK competition with the inhibitor for the binding site with plasminogen. Incubation of plasminogen with cysteine at room temperature for two minutes increased the SK units required for optimum activation to the level at which buffer controls showed inhibitory action. This is valid so long as there was no great excess of inhibitor. After incubation of plasmin with cysteine additional SK did not increase the activity. Therefore, cysteine inhibition is not reversed by SK when urea is not present. In the presence of 1 M urea this competitive phenomenon was more apparent. The addition of urea after incubation with cysteine also shows the activating effect. Urea control was not dependent on the SK units in the range of SK units studied (500 units SK to 5,000 units SK). As cysteine itself adsorbs at 250 mu. the work was eventually extended to include substrate, enzyme and the inhibitor blanks. Artifacts arising from the use of selected conditions have therefore been ruled out. The results emphasize one more similarity of plasmin and trypsin, namely that the reducing agents in concentrations which do not affect the activity of plasmin in the absence of urea are inhibitory in its presence. It is also interesting to note that in studies on trypsin extra kinase has been reported to abolish the inhibition of trypsin by thiol. Further studies of inhibitory action of thiols should be made in vivo. In plasma there has been reported18 to be a 30 mg. per cent of reduced glutathione. Glutathione has also been found in tissues and in red blood cells.

The possibility of still another hypothesis should be considered, namely, the disulfide interchange. The extent to which the —S—S bonds are accessible to glutathione will be determined by the rigidity of protein structure of enzyme, but Green¹⁹ reported heparin and disulfide interchange in plasma clotting by thrombin.

Of interest may be the observation that thrombin caseinolytic activity was not affected by cysteine and mercaptoethanol unless used in urea. No conclusions different from caseinolytic inhibition are drawn regarding the fibrinolysis. The degree of inhibition in a purified plasminogen system is highest when plasminogen is incubated with the inhibitor, followed by activation with small amounts of SK (25 units SK per tube). Spontaneously active euglobulin did not appear to be affected by the incubation time with the inhibitor.

In studies on fibrinolysis the disulfide interchange reaction could explain some of the anomalous results.

SHMMARY

Cysteine and mercaptoethanol inhibit the caseinolytic and fibrinolytic activity of plasmin. Activity losses were uniformly greater in those systems containing 1 M urea. Streptokinase action which is especially pronounced in 1 M urea is discussed.

ACKNOWLEDGMENT

The bovine fibrinolysin and thrombin used in this study were supplied by Parke Davis & Co., Detroit, Michigan; the thrombolysin, streptokinase and human fibrinogen were supplied by Merck Sharp & Dohme, Philadelphia, Pennsylvania.

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Discussion of Paper by Drs. Mootse and Cliffton

DR. GABOR MARKUS (Buffalo, New York): We have found that the use of 2-mercaptoethylamine in a concentration of 0.1 M completely inactivates not only plasmin but also plasminogen. This is not a direct inhibition because the reducing agent can be dialyzed out of the solution completely and the inactivity persists. What this experiment indicates is that mercaptoethylamine acts on plasminogen by reducing a vulnerable disulfide bond which is common to plasmin and plasminogen. We invoke this explanation rather than that of a disulfide exchange because the nitroprusside reaction after dialysis of the reducing agent remains positive.

FROM THE FLOOR: Since the substances that Dr. Mootse deals with are strong reducing agents, is there any evidence that oxidizing agents may reverse the observed effects?

DR. GERDA MOOTSE (New York, New York): We have not tried them.

DR. DANIEL F. KLINE (New Haven, Connecticut): We have also been performing studies with cysteine and mercaptoethanol based on observations Mr. Lassen in Denmark showed me and have been trying to eliminate the plasminogen from fibrinogen solutions by treating them with these reducing agents. We have found that plasminogen activity can be completely removed from fibrinogen solution by treatment with either cysteine or mercaptoethanol but that reoxidation occurs and the plasminogen and proactivator activities return.

The Preparation of Human Urokinase*

J. T. SGOURIS, PH.D., J. K. INMAN, PH.D. and K. B. McCall, Ph.D.

Lansing, Michigan

THE fibrinolytic system has been implicated in the maintenance of the fluidity of blood. Although a great deal of data have been accumulated in the last decade, the exact mechanisms of in vivo fibrinolysis have not been elucidated. The potential value of isolated components of the fibrinolytic system for the treatment of thromboembolic disorders is now recognized.1,2 Whether the most effective thrombolytic and embolytic agent of human origin will be fibrinolysin, urokinase (or other activator), or a combination of these, awaits the preparation of these components in sufficient purity and quantity for large scale evaluation. This goal has been attained in part with our preparation of human fibrinolysin free of extrinsic activator.8

The presence of urokinase in urine was first demonstrated by Williams,⁴ Astrup and Sterndorff⁵ and Sobel et al.⁶ Purification has been achieved by others through adsorption and chromatographic technics.^{7,8} This communication deals with the preparation of the activator, urokinase, for *in vitro* activation of human profibrinolysin.

UROKINASE ISOLATION

Urine was collected (Fig. 1) from normal men, pooled and adjusted to pH 8 with 1 N sodium hydroxide and then cooled to 0° to 2° C. A flocculent precipitate was allowed to settle during a three-hour standing period. The supernate was then removed by decantation and its pH adjusted to 4.5 with 1 N hydrochloric acid. A precipitate was allowed to develop during an overnight standing at 0° to $+2^{\circ}$ C. after a preliminary cooling to about -3° C. The precipitate was recovered by continuous flow centrifugation at 0° C. and $13,000 \times g$.

Further purification was achieved with a barium sulfate adsorption technic similar to that employed by von Kaulla⁸ with fresh urine. This precipitate was redissolved in distilled water to 5 per cent of the original urine volume. The solution was made alkaline (pH 8.5 ± 0.1) with 1 N sodium hydroxide

and stirred with freshly prepared barium sulfate (1 gm./L. of urine volume) for at least fifteen minutes at 0° to + 5°c. The barium sulfate and adsorbed materials were recovered by batch-type centrifugation and resuspended in 5 per cent of the urine volume of distilled water at 0° to +5°c. in order to wash out possible thromboplastic activity. The washed barium sulfate was centrifuged, and the urokinase activity was eluted by washing the precipitate twice with 3 per cent of the urine volume of 2 per cent (w/v) sodium citrate (dihydrate). The citrate was removed from the combined eluates by adding 3 M of barium chloride for each 2 M of citrate ion present (0° to +5°c.). The suspension was centrifuged, and the precipitate (barium citrate plus adsorbed impurities) was discarded. supernate was adjusted to pH 4.5 with 1 N hydrochloric acid and 95 per cent ethanol was added to a concentration of 20 per cent (v/v). The temperature was allowed to fall from 0° to -5° c. during the addition of ethanol. The precipitate, containing urokinase, was removed by centrifugation at −5°c. and redissolved in distilled water to 2 per cent of the original urine volume and adjusted to pH 8. An excess of disodium ethylenediaminetetraacetate (EDTA) was added to chelate the remaining barium. Approximately 20 ml. of 0.2 M EDTA was added to each liter of solution and the pH was adjusted to 8 with 1 N sodium hydroxide. The solution was then dialyzed thoroughly against cold distilled water and dried from the frozen state. The recovery was in the range of 30 to 40 per cent of the original activity.

ANALYTICAL METHODS

Urokinase was assayed by a modification³ of the Remmert and Cohen⁹ caseinolytic assay. This assay is based on the ability of urokinase to activate highly purified profibrinolysin (excess substrate) in a given period of time. The potencies of these preparations are in the range of 1,800–2,100 caseinolytic units per milligram nitrogen.

UROKINASE PROPERTIES

Thromboplastic Activity: No direct case in olytic activity could be detected with this preparation.

^{*} From the Division of Laboratories, Michigan Department of Health, Lansing, Michigan. This work was performed at the request of the Director of the American National Red Cross Blood Program under an agreement between the American National Red Cross and the Michigan Department of Health Laboratories.

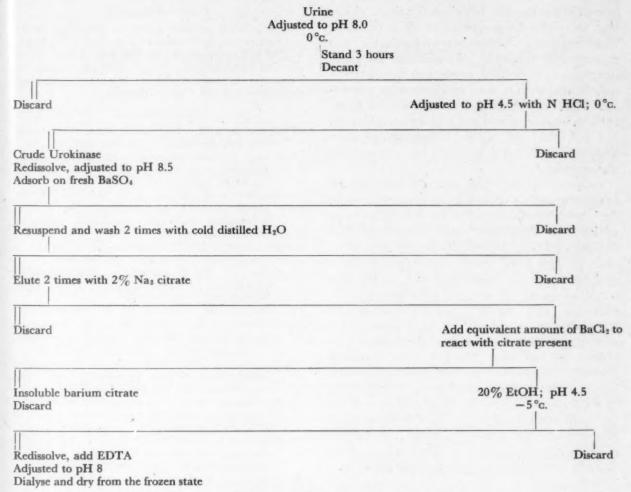


Fig. 1. Isolation of urokinase.

No fibrinolytic activity could be detected on heated human fibrin plates.¹⁰ However, urokinase, like streptokinase, could lyse the fibrin in the unheated human fibrin plates because of the presence of profibrinolysin in the human fibrinogen used to prepare the plates. The urokinase was tested by a recalcified clotting time test and was found to contain no detectable thromboplastic activity.

Uropepsin Activity: The instability of urokinase at acid pH's has been attributed to the proteolytic activity of uropepsin originating from the chief cells of the gastric mucosa. The presence of proteolytic activity at an acid pH was determined on human albumin plates. These plates were prepared as follows: Five milliliters of a 1.2 per cent Difco agar prepared in a pH 1.8, $\Gamma/2$ 0.1 glycine hydrochloride buffer was mixed with 5 ml. of 0.5 per cent human albumin in the same buffer. The agar was allowed to set and single drops (0.08 ml. each) of the test sample were placed

on the plates and incubated at 32°c. for up to eighteen hours. The reaction was stopped by flooding the plates with 10 per cent trichloracetic acid. This resulted in good contrast between the lysed and unlysed areas. Pepsin, as well as urine, adjusted to pH 1.8 showed proteolytic activity on these plates. Addition of pepsin to urine showed no change in proteolytic activity toward the albumin plates; however, adjusting the pH of the urine containing pepsin to 8 for ten minutes resulted in total loss of proteolytic activity. These conditions are similar to the first step of urokinase preparation; thus our purification procedure probably destroys any uropepsin present, for no proteolytic activity can be detected in our final preparation.

Viral Contamination: In the preparation of a clinically acceptable product, the problem of viral contamination had to be considered. We have used two methods for reducing the risk of transmitting viral disease (particularly

hepatitis B) in our blood derivatives, either heat treatment for ten hours at 60°c. or ultraviolet radiation. Of the two methods, the heat treatment is preferred when it can be used. Attempts to determine conditions that permitted the heat treatment of urokinase were successful. Heat treatment of urokinase for ten hours at 60°c, was conducted in 0.3 M sodium chloride, pH 6.7, 0.01 M sodium phosphate buffer. Sixty-five to 75 per cent of the original activity was recovered.

Enzymatic Action: The optimal pH for the enzymatic action of urokinase on profibrinolysin¹² was found to be 7.4 to 7.6. However, purified profibrinolysin and fibrinolysin had limited solubility and stability in aqueous buffers near neutral pH. Since Alkjaersig, Fletcher and Sherry¹³ had shown that pH 7.6 aqueous natural glycerol (50 per cent v/v) increased the solubility and stability of fibrinolysin, we explored the possibility of activating heattreated profibrinolysin with heat-treated urokinase in buffered, 50 per cent synthetic glycerol. Activation proceeded enzymatically in this medium, and because of the high stability of the formed fibrinolysin, large amounts of the profibrinolysin could be activated with but small amounts of urokinase by allowing the reaction to proceed for many days at 32°c. The aqueous glycerol served also as a bacteriostatic medium.

SUMMARY

A highly potent preparation of urokinase has been separated from human urine and has been successfully heat-treated for ten hours at 60°c. It is free of thromboplastic activity and is non-pyrogenic. This preparation was successfully used to prepare fibrinolysin from human, heat-treated profibrinolysin. Thus it is possible to make available preparations containing heattreated proteins of only human origin for evaluation in the dissolution of spontaneous thromboses in man

ACKNOWLEDGMENT

Arrangements for urine collection at the State Prison of Southern Michigan were made by N. D. Henderson, M.D. of our Laboratories and Mr. Jack White, Prison Hospital Administrator. We gratefully acknowledge the advice and suggestions of L. A. Hyndman, Ph.D., and the assistance of Mr. Thomas Bartshe.

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The Biochemistry and Physiology of Urokinase*

DAVID R. CELANDER, PH.D. and M. M. GUEST, PH.D.

Galveston, Texas

Our interest in urinary factors developed somewhat prior to 1952 in which year we, together with Astrup and Sterndorff, independently arrived at the same conclusion as had Williams that there existed in human urine an activator, capable of converting human profibrinolysin to fibrinolysin; this activator we termed urokinase. We have since directed attention to the development of methods for purification and assay of urokinase, for the elucidation of its biochemical properties and to its physiologic significance. Furthermore, urokinase has been and is being employed as a tool in investigating the physiologic role of the fibrinolytic system.

PURIFICATION AND ASSAY

pH 4.5 Precipitate: Our early efforts to concentrate urokinase were rewarded by the discovery that it was nearly quantitatively (90 to 100 per cent) removed from human urine in active form adsorbed to the precipitate which deposits when the urine is adjusted to pH 4.5 and frozen. However, the activity could not be separated from this precipitate by simple extraction even by concentrated solutions of urea, potassium thiocyanate or dilute sodium hydroxide. In 1953 we succeeded in obtaining soluble concentrates from this precipitate by dialysis of its suspension at room temperature against saturated sodium tetraborate for periods of three to four days. The solution of urokinase was apparently related to the slow dissolution of the precipitate followed by removal of the dissolved crystalloids during the prolonged dialysis. Subsequent to this we observed that treatment of the precipitate with 2 per cent EDTA at pH 6.8 removed color and impurities and that adjustment of the pH to 8.6 led to the extraction from the precipitate of 25 to 40 per cent of the bound urokinase in soluble form.

Assay Systems. Development and Validation: With soluble concentrates of urokinase available, attention was directed to the development of adequate methods for its assay. At the time of its discovery there were no technics for reliably estimating urokinase. We knew only that whole urine and concentrates of urokinase could bring about the conversion of the profibrinolysin of several species to fibrinolysin and that these same preparations could bring about the lysis of clots formed from "purified" thrombin and fibrinogen. Whether or not the two actions were both due to a kinase alone or to a kinase plus a protease was not known. Urokinase solutions, added to clots formed by the treatment of 0.1 per cent freeze-thaw bovine fibrinogen with Parke-Davis thrombin, gave lysis times (tilt-tube method11) at 37°c. which, when plotted logarithmically against the urokinase concentrations employed, resulted in a straight line. With this one-stage technic it appeared possible to quantitatively estimate the concentration of urokinase in a solution.

At the time these experiments were carried out, it was not recognized that bovine fibrinogen or thrombin contained profibrinolysin since bovine fibrin clots were resistant to lysis by streptokinase, an activator which quickly brought about the dissolution of the clots of man and some other species. To validate the one-stage assay procedure, a two-stage procedure was developed. In the first stage an arbitrarily chosen excess of human profibrinolysin was incubated with varying concentrations of urokinase for a fixed length of time (thirty-five minutes) at 28°c. in a system buffered with imidazole or 0.067 M phosphate buffer at pH 7.25. In the second stage, the lysis times of 0.2 ml. aliquots were determined in a 0.4 ml. system containing 0.1 per cent bovine fibrinogen clotted with Parke-Davis thrombin, pH 7.25, at 37°c. When the lysis times of these clots were plotted logarithmically against the concentration of urokinase, a straight line was obtained. Since activation continued during the period of active lysis,

^{*} From the Department of Biochemistry and the Carter Physiology Laboratory, University of Texas Medical Branch, Galveston, Texas. This work was supported in part by a contract between the United States Air Force School of Aviation Medicine, Brooks Air Force Base, Texas and the University of Texas Medical Branch, Galveston, Texas (Contract AF 41 (657)-228), by U. S. Public Health Service Grant A-557(C6), and by the James W. McLaughlin Fund for the Study of Infection and Immunity, at the Medical Branch.

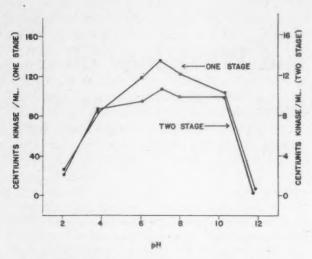


Fig. 1. pH stability of urinary kinase activity. Onehalf milliliter aliquots of urokinase solution were adjusted to the pH indicated by mixture with 0.5 ml. of the appropriate buffer and incubated at that pH for fortyfive minutes at 37°c. Buffer systems used: citrate-NaOH-HCl, pH 2.1 and 3.8; Na₂HPO₄-citric acid, pH 6.15; Na₂HPO₄-KH₂PO₄, pH 7 and 8; NaOH-NaClglycine, pH 10.3 and 11.9. Ionic strength of all buffers, approximately 0.1. pH of all systems was returned to 7.25 by dilution with two parts pH 7.25 imidazole buffer prior to assay by the one-stage (Fig. 4) and two-stage methods. Incubation mixture of the twostage method consisted of 1 ml. of solution under test in which was dissolved 3 mg. of protamine precipitated human profibrinolysin.11 This mixture was incubated at 28°c. for thirty-five minutes; 0.2 ml. aliquots of the incubation mixture were withdrawn and added to 0.2 ml. 0.2 per cent bovine fibrinogen and clots formed by addition of 0.005 ml. bovine thrombin (1,400 units per ml.) and allowed to lyse at 37°c. The two-stage unit is defined in terms of the amount of kinase activity needed to generate one unit of fibrinolysin11 under the conditions described in the text, and is equal to approximately ten one-stage units.

this procedure was not ideal but we were able to demonstrate that it measured activator primarily. The one-stage and two-stage assays were subsequently compared and found to give similar results when employed in the testing of urokinase under a wide variety of circumstances. The activity which each measured was shown to be stable at pH's ranging from 4 to 10 (Fig. 1); to be relatively resistant to heating at a neutral pH (Fig. 2); and to concentrate in a typical pattern in foam fractions collected from human urine (Fig. 3). Furthermore, employing the technic of Müllertz12 it was observed that profibrinolysin, the presence of which was required so that the one-stage assay could work, was present in the freeze-thaw bovine fibrinogen used in the assay. On the basis of such studies we concluded in 1954 that the simpler one-stage procedure, in which urokinase was mixed directly with freeze-thaw bovine fibrinogen immediately before clotting with Parke-Davis thrombin, was adequate for use in the measurement of uro-

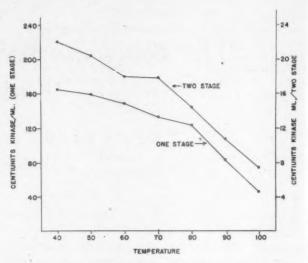


Fig. 2. Heat stability and urokinase. Twelve units (1,200 centiunits) of urokinase solution contained in 1 ml. 0.067 M phosphate buffer, pH 7.25, were incubated at the temperatures indicated for fifteen minutes, chilled, appropriately diluted and assayed by the one- and two-stage assay methods as previously described (Fig. 4 and legend of Fig. 1).

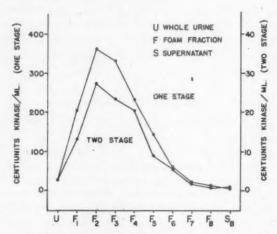


Fig. 3. Distribution of urinary kinase activity on foam. Air was bubbled into 500 ml. of pooled urine placed in a 1 L. cylinder closed by a rubber stopper perforated by a bubbler tube and by a delivery tube from which foam fractions were collected for assay by the one- and two-stage technics previously described (Fig. 4 and legend of Fig. 1).

kinase in the absence of appreciable amounts of active protease.

The description of the assay and the definition of the urokinase unit were published in 1955.⁵ Figure 4 depicts the relation between centiunits (one-hundredth unit) of urokinase activity and lysis time in seconds of our standard 0.4 ml. clot. Although our unit of urokinase activity is based on lysis time of a 1 ml. clot, the 0.4 ml. clot has been employed routinely in order to conserve reagents. It was established that the dilution curves of urokinase were

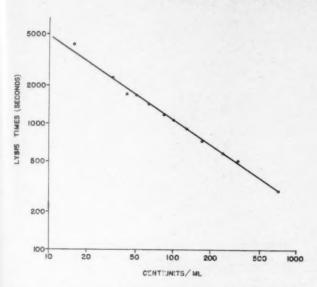


Fig. 4. One-stage assay for urokinase. Reaction mixture: 0.2 ml. 0.2 per cent bovine freeze-thaw fibrinogen, 0.2 ml. whole urine (previously dialyzed against fifty volumes of 0.067 M phosphate buffer, pH 7.25, twenty hours) or purified urokinase preparations made in the same buffer or buffer of equivalent ionic strength and pH. Clotted with 0.005 ml. bovine thrombin (Parke-Davis, 1,400 units per ml.). Lysis times determined by tilt-tube method¹¹ at 37°c. and converted to centiunits per ml. of solution assayed by reference to dilution curve for 0.4 ml. system.

parallel whether the 1 ml. or 0.4 ml. system was used. Therefore, by use of appropriate constants, activity against a 0.4 ml. clot could be expressed in terms of the unit based on lysis time of a 1 ml. clot. The onestage assay functions best at lysis times between ten and seventy minutes. Longer lysis times tend to be less accurate while shorter lysis times are undesirable since the profibrinolysin present in the reagents may become limiting. No fibrinogen and thrombin preparation should be employed which produces a clot which has a lysis time longer than three minutes in the presence of a concentrated preparation of activator (100 units per ml. or more of urokinase). In addition no fibrinogen and thrombin preparation should be employed which produces a clot with a control lysis time of less than forty-eight hours.

Urokinase Units: For comparative purposes we have related the expressions of unit activity used by other workers to our one-stage unit definition. By using the bead assay method of Ploug and Kjeldgaard¹³ with our urokinase and by assaying their preparations by our one-stage method, we have found that our unit is equal to approximately ten of the Leo units¹³ and approximately eighty of the units employed by Smyrniotis et al.¹⁴ Direct comparison of our unit with that of von Kaulla¹⁵ has not been made. However, by comparing the urokinase unitage of normal human urine as measured by our one-stage assay and as reported by him, ¹⁵ it appears

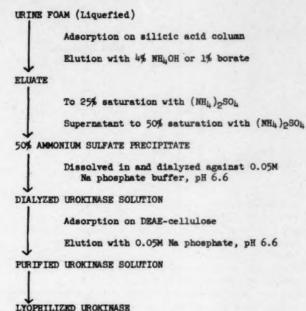


Fig. 5. Preparation of urokinase. All liquefied urine foam is adjusted to pH 9 (1 N NaOH) at room temperature and kept overnight in a cold room to permit separation of inert materials (primarily phosphates) and freed of these as well as attendant insoluble materials by centrifugation at 25,000 × g. Prior to silicic acid chromatography the pH is adjusted to 7 (1 N HCl). Procedure employs 1 gm. silicic acid per 2,000 units of urokinase activity to be adsorbed. DEAE-cellulose columns prepared as described in section on purification of thrombin. For the chromatography of a given amount of urokinase, the amount of DEAE-cellulose must be determined empirically and varies from one lot of DEAE-cellulose to another.

that ten of our units are equal to one of the von Kaulla units.

Foam Procedure: Our observation that urokinase concentrated in the foam which formed when human urine was shaken led to the development of a foaming procedure in which 80 per cent of the urokinase activity present in urine could be concentrated in 5 per cent of the original volume. The activity was further concentrated by precipitating liquefied foam at pH 4.5 and brought into solution by extracting the precipitate at pH 8.6 with EDTA as previously described for whole urine. However, since losses were great under these conditions, we abandoned the 4.5 procedure in 1955. Other adsorbents, including barium sulfate, calcium carbonate, tricalcium phosphate and IRC-50, were tested and found unsatisfactory either because they failed to adsorb the urokinase or because urokinase once adsorbed could not be satisfactorily eluted from them.

Further studies in purification were materially augmented by the observation of Ploug and Kjeldgaard¹⁶ that urokinase could be quantitatively adsorbed on silicic acid. This technic applied to liquefied urine foam provided us with the first relatively large

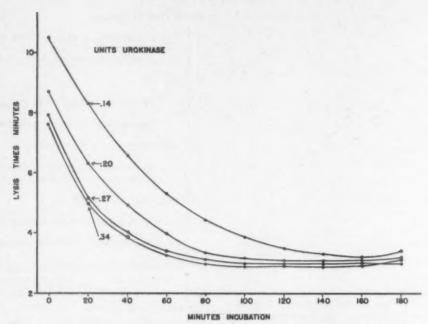


Fig. 6. Conversion of profibrinolysin to fibrinolysin by urokinase. Incubation system: 1 ml. purified human profibrinolysin containing 12 mg. of preparation described in legend of Figure 1; varying quantities of urokinase solution, and buffer, pH 7.25, to a total of 4 ml. Units of urokinase shown indicate number of two-stage units (legend of Fig. 1) per ml. incubation mixture. At times indicated 0.2 ml. aliquots were withdrawn and the amount of fibrinolysin present was determined.

quantities of urokinase solutions which were sufficiently concentrated to employ, with economy and satisfaction, conventional purification technics such as ammonium sulfate fractionation and chromatography. Our method, in which urokinase is concentrated preliminary in foam, is described in Figure 5. Chromatography on diethylaminoethyl (DEAE)-cellulose columns at pH's from 6 to 8 removes most of the impurities and all of the remaining color but permits the passage of urokinase without changing either the pH or the ionic strength. A repeat of the DEAE-cellulose step gives excellent preparations with specific activity approaching 6,000 of our units per mg. protein. The final preparations provide from 0.1 to 0.3 mg. protein per L. of urine processed.

PROPERTIES

In 1955 we reported the properties of the partially purified urokinase we had prepared. It was a water-soluble protein, which retained its activity between pH's 4 and 10 and possessed considerable resistance to denaturation by heat, being only 50 per cent destroyed at pH 7.25 when incubated at a temperature of 80°c. for fifteen minutes. Its action in the conversion of profibrinolysin to fibrinolysin is illustrated in Figure 6. The total amount of fibrinolysin generated was independent of the urokinase concentration. However, the rate of the reac-

tion depended on the concentration of urokinase. When the rate of conversion of profibrinolysin to fibrinolysin in the second twentyminute period of incubation was plotted arithmetically against the concentration of urokinase present, a straight line relation was obtained (Fig. 7). On the basis of these findings we concluded that urokinase effected the conversion of profibrinolysin to fibrinolysin enzymatically and that the conversion proceeded essentially by first order kinetics. Since we had been able to demonstrate that urokinase was weakly proteolytic against casein, we thought that some kind of proteolysis might be involved in its activation of profibrinolysin.

Our findings regarding the enzymatic character of urokinase were confirmed in 1956 by Sgouris¹⁷ employing somewhat different technics and urokinase isolated by our earlier procedures. Additional reports on the enzymatic nature of the urokinase activation of profibrinolysin were made by Sherry and Alkjaersig¹⁸ and by Ploug and Kjeldgaard^{16,19} with their highly purified preparation of urokinase in 1956 and 1957. In addition, the latter workers¹⁹ clearly demonstrated that the pH optimum for the reaction was 9 and that urokinase brought about the proteolysis of protamine-heparin

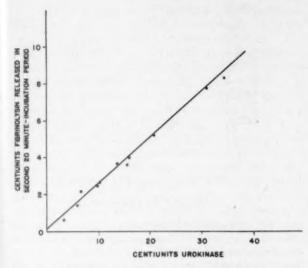


Fig. 7. Effect of concentration of urokinase on rate of conversion of profibrinolysin to fibrinolysin. Urokinase concentrations expressed in terms of one-hundredth of the urokinase two-stage unit. Fibrinolysin generated expressed in terms of 0.01 Guest units.¹¹

complexes and the hydrolysis of esters of arginine and lysine. We have recently confirmed their findings regarding the ability of urokinase to bring about the hydrolysis of tosylarginine methyl ester (TAMe) and of lysine ethyl ester (LEe). However, using the method of Roberts20 to measure the rates at which equal concentrations of urokinase effect the hydrolysis of TAMe and LEe, we find the rate of hydrolysis of TAMe relative to the rate of hydrolysis of LEe considerably higher than that reported by Kjeldgaard and Ploug.19 These authors also demonstrated that the apparent instability of urokinase at acid pH was due to the presence, in urine and urokinase preparations, of a pepsin-like enzyme which brought about its destruction in the presence of acid. We have confirmed these findings but must point out that the presence of pepsin in urokinase preparations may be masked if the solutions of urokinase tested are too concentrated. We have been able to detect traces of this urokinase-destroying activity at pH 3 in most of our urokinase preparations and in all of the Leo preparations which we have had the opportunity to test.

EXCRETION

Errors Involved in Excretion Determinations: During the past several years we have accumulated information regarding patterns of excretion of urokinase and can report that in general our data agree with those published by Bjerrehuus,²¹ von Kaulla²² and Smyrniotis et al.¹⁴

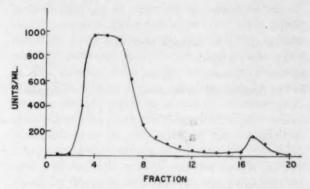


Fig. 8. Fractionation of urokinase on DEAE-cellulose, Activity of dialyzed urokinase solution (Fig. 5) is determined and a column containing approximately 1 mg. DEAE-cellulose per 100 units urokinase to be chromatographed is equilibrated with 0.05 M phosphate buffer, pH 6.6. Urokinase solution is carefully poured on to column, followed immediately by five 20 ml. batches of the same buffer. Eluate collected in 5 ml. fractions which were diluted and assayed for urokinase activity by the one-stage method (Fig. 4).

Publication of most of our findings has been withheld primarily because of several incompletely resolved theoretical considerations. First, we have observed the presence of materials in urine which inhibit the action of urokinase at neutral pH. The quantitative recovery of added urokinase from urine does not afford adequate evidence that such materials are absent. Second, during our extensive use of DEAE-cellulose in the purification of urokinase we have observed elution patterns similar to that depicted in Figure 8. Here two discrete peaks were obtained, both of which possessed urokinase activity. Results of this nature tend to suggest that more than one kinase may be present in urine and that up to the DEAEcellulose step, one deals with a population of molecules sufficiently similar to traverse the steps of purification together, their slight dissimilarity appearing only when a chromatographic material of high resolving power is employed. Since the chromatographs were obtained from urokinase isolated from large samples of pooled human urine, it is not possible at this time to state whether both types of activity occur in the same person or whether they were supplied by different persons. Analysis of the material contained in these peaks revealed moderate variation in the slope of dilution curves. The slopes differed sufficiently to introduce a large error should one or the other of these types of urokinase predominate in the urine under analysis.

Significance of Variations in Excretion: Experi-

ments we have performed in the dilution of urine itself prior to or following dialysis and the testing of the diluted samples have revealed that not infrequently urine samples do not dilute as predicted by an assay curve. Thus, it is our current belief that a degree of reservation must be attached to data regarding variability in urokinase excretion. Of particular importance as pointed out by von Kaulla,22 changes in the excretion pattern on the part of a person whose pattern is known constitute data of probable value, whereas isolated findings, on the basis of which attempts to decide whether a person's urokinase excretion is normal or abnormal, are perhaps of less value at the present time. However, as more data accumulate, this problem will be subject to resolution and indeed the data presented by Smyrniotis et al.14 afford evidence that at least a part of the problem has been resolved.

Dietary Salt Intake: It is with reservation that we present certain of our findings with regard to the effect of levels of salt in the diet and of exercise on urokinase excretion. In experiments lasting for three days, medical students were placed on diets with severely restricted salt content, on conventional diets of moderate salt content, and on diets in which approximately 10 gm. of additional salt were supplied per day. It was observed that urokinase excretion in the first group averaged 814 units per day while that of the second was 423 units and that of the third was 370 units in the twenty-four hour samples collected on the third day. Additional studies of this nature are still in progress.

Exercise: Exercise on a treadmill under controlled conditions with the subject operating at approximately 75 per cent of capacity appears to produce an increase in urokinase excretion and, without exception, produces an increase in plasma fibrinolytic activity and plasma activator. In these experiments, subjects ranging in age from nineteen to seventy-eight years have been tested; the data are preliminary but those relating to blood fibrinolytic factors are in essential agreement with those obtained by others.23-25 On the basis of our limited observations, urokinase excretion of persons on a daily exercise schedule during and immediately following the exercise period tends to show a progressive rise.

Species Variations in Response to Urokinase We have found human urokinase capable of ac-

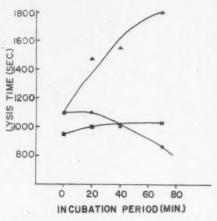


Fig. 9. Adsorption of urokinase inhibitors from pig globulin. Pig plasma diluted with nineteen volumes of distilled water and adjusted to pH 5.5 with 0.1N H₂SO₄. Euglobulin precipitate was collected, washed twice, and dissolved in 0.067 M phosphate in an amount equal to half the original plasma volume. This constitutes the non-adsorbed globulin preparation (triangles). Five milliliters of the globulin solution was throughly mixed with a paste supplying 50 mg. of magnesium hydroxide and centrifuged. The supernatant was reprecipitated by dilution and adjustment to pH 5.5 and the globulin precipitate was dissolved to a volume of 5 ml. in 0.067 M phosphate buffer. This preparation constitutes the adsorbed globulin (circles). One-half milliliter of each preparation was mixed with 25 units of urokinase contained in 1 ml. and with 0.5 ml. buffer, pH 7.25. The control system (squares) was comprised of 25 units of urokinase in 1.0 ml. plus 1.0 ml. buffer, pH 7.25. At the times specified, aliquots were withdrawn, diluted 1:10 with phosphate buffer and lysis time determined as in the second stage of the two-stage assay.

tivating the profibrinolysin of a number of mammalian species including man, cat, rat, cow, rabbit and dog. In addition, urokinase preparations derived from the urine of these species and of the hamster have been found to bring about varying degrees of activation of the plasma profibrinolysin of the species against which they were tested. Recently, human urokinase was found to effect activation of the profibrinolysin of the mullet, a species of fish.

On initial examination in 1953, the pig appeared to present a glaring exception to these findings in that globulin fractions prepared from pig plasma were refractory to activation by urokinase. Clots formed by mixing pig fibrinogen and bovine thrombin were only weakly proteolyzed in the presence of urokinase (requiring the use of 50 per cent more kinase and clots only one-fourth the strength of corresponding bovine clots to obtain acceptable lysis times by the one-stage method). When pig thrombin isolated by the method of Seegers et al.26 was employed to coagulate the pig fibrinogen, extreme resistance to lysis by urokinase was observed. Since pig profibrinolysin, if present at all in either pig plasma or pig fibrinogen, appeared to be incapable of activation by urokinase, it was thought that clots formed from pig fibrinogen

and pig thrombin could be used to differentiate fibrinolytic activity supplied by an exogenous source from fibrinolytic activity initiated within the clot itself through the activation of endogenous profibrinolysin by exogenous kinases. However, to be certain of the validity of the procedure it was necessary to investigate, first, the susceptibility of the pig fibrin to known proteases, and second, the ability of pig profibrinolysin to be activated.

It was found that clots formed by treatment of pig

fibrinogen with pig thrombin were also more resistant to proteolysis by fibrinolysin and trypsin than were clots formed from bovine reagents, a resistance which could not be demonstrated as due to an excess of antifibrinolysin. In the course of these studies, it was also found that clots formed from pig fibrinogen with increasing amounts of Parke-Davis thrombin topical in the presence of a constant amount of urokinase gave lysis times which were proportional to the amount of thrombin added, thus confirming beyond question our suspicions that commercial bovine thrombin provided a liberal addition of profibrinolysin to that already present in the bovine fibrinogen of our assay system. Siegel and Cliffton²⁷ demonstrated conclusively the presence of profibrinolysin in thrombin of commercial origin and outlined a method for

its removal by starch electrophoresis.

The problem of activating pig profibrinolysin still remained. As previously noted, fractions of pig thrombin which had been prepared by the method of Seegers26 enhanced the resistance of pig fibrin clots to urokinase activity. This enhancement was beyond that which could have been expected through omission of the profibrinolysin presumed to be supplied by commercial bovine thrombin. Since magnesium hydroxide is used in Seeger's method for the preparation of thrombin to adsorb prothrombin from plasma, pig plasma and plasma fractions were adsorbed with magnesium hydroxide and the supernatants were tested for their ability to be activated by urokinase of human origin. It was found that following adsorption with magnesium hydroxide not only the plasma globulin fractions obtained from pig plasma but also pig plasma itself developed fibrinolytic activity in the presence of urokinase (Fig. 9). Thus it was established that pig plasma did contain profibrinolysin the activation of which by urokinase is prevented by a substance adsorbed by magnesium hydroxide. Pig fibrinogen, as a possible solution to the problem of measuring proteolytic activity in the presence of activator, was abandoned in 1954.

PURIFICATION OF THROMBIN AND FIBRINGGEN

In the course of our studies of the properties of the system of the pig, we became aware of the capabilities of human urokinase as a tool in the detection of profibrinolysin in the various reagents needed for study of the fibrinolytic enzyme system and have since made extensive

use of it, for example, in the purification of bovine fibrinogen and bovine thrombin. Preliminary results described in 195910 have been extended and we have shown that it is possible by chromatography on DEAE-cellulose at neutral pH to separate from thrombin the contaminating profibrinolysin which invariably accompanies the commercial preparations. A single chromatographic step will serve to illustrate this (Fig. 10). Freeze-thaw human fibrinogen relatively free of profibrinolysin was incubated with fractions taken from a column (7.5 cm. high × 32 mm. ID*) containing 5 gm. of DEAE-cellulose which had been charged with 2 ml. of a solution of thrombin (1,000 units per ml.) in 0.05 M phosphate buffer and then eluted as described in Figure 10. There is a considerable distance between the peak of thrombin and the peak of profibrinolysin and a relatively good separation can be achieved with a single chromatographic purification. Subsequently we have found that a repeat of this step results in the removal of sufficient profibrinolysin to render the thrombin comparable (with respect to profibrinolysin contamination) to that supplied to us by Seegers, whose preparations were found free of profibrinolysin when tested by our technics. The fibringen employed in these tests (Fig. 11) was a freeze-thaw preparation of human fibrinogen, the minimum lysis time of which, in the presence of optimum concentration of kinase (streptokinase-activated proactivator or urokinase), was approximately thirty minutes.

We have subjected fibrinogen to a variety of treatments. In the case of bovine fibrinogen, treatment with approximately 20 to 25 mg. calcium phosphate gel per ml. of 0.2 per cent fibrinogen at neutral pH resulted in a supernatant solution of fibrinogen which was carried through various concentration procedures and diluted with imidazole buffer, pH 7.25, to a final 0.2 per cent concentration. When this fibringen solution was clotted with profibrinolysin-free thrombin in the presence of urokinase the clots formed did not lyse in seven days. The urokinase used in this test brought about a 5-minute lysis time in a standard clot containing profibrinolysin which was supplied both by the thrombin and by the fibrinogen. We believe that fibringen of this quality is adequate to permit the differentiation of kinase and protease activity. The over-all yields in such

^{*} Inside diameter.

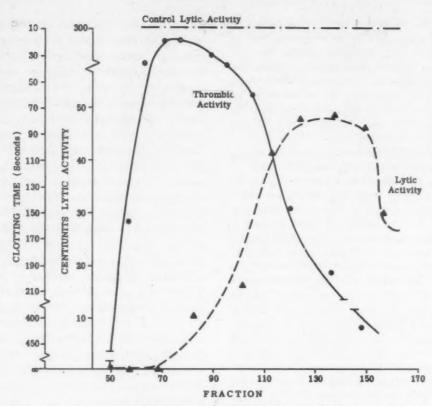


Fig. 10. Chromatographic separation of profibrinolysin from commercial Thrombin, adsorbed on a DEAE-column equilibrated with 0.05 M phosphate buffer, pH 7, was eluted sequentially by 0.05 M phosphate buffer, pH 7, 0.1 M sodium dihydrogen phosphate, and finally by 0.5 M sodium dihydrogen phosphate, pH 4.7. The eluate was collected in 0.5 ml. fractions. Every fifth fraction was assayed for thrombin activity and profibrinolysin. Thrombin assay system: 0.006 ml. thrombin eluate fraction, 0.2 ml. 0.067 M phosphate buffer, pH 7.2, and 0.2 ml. 0.2 per cent bovine fibrinogen. Clotting time determined at 37°G. Lytic assay system: 0.2 ml. human fibrinogen, 0.2 per cent; 0.05 ml. 1:3,200 human plasma (proactivator source); 0.1 ml. streptokinase (1,000 units per ml.); 0.05 ml. thrombin eluate fraction; 0.005 ml. Seegers' bovine thrombin (200 units per ml.). Lysis times at 37°c. converted into centiunits of lytic activity; i.e., one-hundredth unit of fibrinolysin. This system was employed in preference to one containing urokinase because streptokinase in the concentration indicated prevents the development of fibrinolytic activity from small amounts of profibrinolysin present in the human plasma or human fibrinogen. In the control lytic system, the fibrinogen and thrombin are supplied as untreated bovine preparations.

preparations range from 12 to 20 per cent and occasionally are as high as 50 per cent. Since the procedure has been successfully employed not only on freeze-thaw fibrinogen but also on commercial fibrinogen (Armour), it appears to be an economical one and may afford a substitute for the heated fibrin plates which have several drawbacks, including a decreased sensitivity to proteolysis.²⁸

ANTIGENICITY OF UROKINASE

Recently we obtained evidence that the urokinase isolated from one species will act as a potent antigen in another. Guinea pigs immunized to human urokinase in amounts as small as 180 gamma urokinase protein will respond with anaphylactic death to a challenge of as little as 75 gamma. Such animals have been employed to demonstrate the passage into urine of the rabbit of human urokinase administered intramuscularly. A total of 3,000 units of human urokinase (1.25 mg. protein) was administered in seven divided doses of 1 ml. each over a period of five weeks. The urine was collected and the urokinase isolated. It was found that: (1) Following injection of human urokinase there was a precipitous increase in the excretion of urokinase. (2)

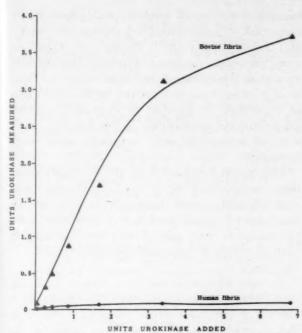


Fig. 11. Sensitivity to urokinase of clots prepared from bovine fibrinogen and human fibrinogen. *Bovine system:* 0.2 ml. bovine fibrinogen, 0.2 per cent; 0.2 ml. urokinase dilution; 0.005 ml. Parke-Davis thrombin (280 units per ml.). *Human system:* 0.2 ml. freeze-thaw fibrinogen, 0.2 per cent; 0.2 ml. urokinase dilution; 0.005 ml. Seegers' thrombin (200 units per ml.). Lysis times observed at 37°c., converted to units of urokinase from the one-stage assay curve (Fig. 4) and plotted against units of urokinase known to have been added.

Preparations of this urokinase fraction obtained from rabbit urine by the technics already described for the isolation of human urokinase (Fig. 5) produced anaphylactic death when supplied to guinea pigs previously sensitized to human urokinase. Urokinase excretion in noninjected control rabbits studied during a similar period was relatively constant and preparations of this urokinase produced no detectable symptoms in guinea pigs immunized to human urokinase: These data are interpreted as an indication that human urokinase passed into the blood stream from the site of injection (muscle), was transported to the kidney, and crossed the rabbit's glomerulus, presumably retaining not only its enzymatic properties, as indicated by the enhanced urinary activity, but also its immunologic characteristics. These results were obtained with a heterologous preparation of urokinase supplied exogenously to which, as will be noted later, inhibitors are formed. Therefore, it appears that conditions would be even more favorable for the passage of activators elaborated in the tissues or blood of the animal itself into the urine of that animal,

Table I

Antikinase in the Serum Globulin of Urokinase-Sensitized Rabbits

Source of Serum Globulin	Urokinase (units)
Saline control	92
Rabbits injected with a total of 720 µg. urokinase protein in divided doses	48
Rabbits injected with a total of 900 µg.	
urokinase protein in divided doses	12
Uninjected rabbits	101
Rabbits injected with 3 mg. egg albumin	
in divided doses	132

Note: Plasma globulin fraction precipitated by 36 per cent saturation with ammonium sulfate, dialyzed against 0.067 M phosphate buffer, pH 7.25, incubated with 92 units urokinase for 30 minutes at 37°C., appropriately diluted and assayed for residual urokinase by the one-stage method (Fig. 4).

such activator activity being identified as urokinase. von Kaulla²² has reported that alterations in the fibrinolytic activity of blood and plasma are related to alterations in urokinase excretion. Thus the suggestion that urokinase is of systemic origin, possibly at sites other than the kidney, does not appear to be untenable.

We have found that the plasma of several species contains inhibitors to urokinase activity. The formation of apparently specific inhibitors to heterologous urokinase has been demonstrated in the rabbit. From the data of Table I it can be reasonably concluded that in a rabbit subjected to a standard immunologic induction with urokinase potent inhibitors to urokinase develop in its plasma globulin fraction. In experiments of this type special precautions were taken to avoid the effects of activated profibrinolysin by employing relatively large amounts of urokinase and then diluting the samples prior to testing.

Nature of Antibodies to Urokinase: Experiments are in progress to determine the nature of the antibodies produced to urokinase not only in terms of their effect upon urokinase but also with respect to their specificity. In all experiments with preparations of Leo urokinase, anaphylactic death was produced in guinea pigs immunized to our preparations of human urokinase. Furthermore, all of their preparations, when tested by the Ouchterlony agar diffusion²⁹ technic against rabbit serum globulins isolated from rabbits immunized to our urokinase,

exhibited one and often more than one band of precipitation. We are relatively certain that at least one of these bands is due to urokinase itself. However, since the preparations we have employed to achieve immunization of our rabbits are not homogeneous, definitive evidence is not yet available that more than one antigen having urokinase activity is excreted by an animal of any species.

COMMENTS

Our experimental approach to the study of urokinase can be summarized in the statement that our goal has been and is a better understanding of the biochemistry and physiology of the fibrinolytic enzyme system. We have not directed our efforts toward the mass production of commercial quantities of urokinase or to its in vivo testing in the human being, but, with the facilities available to us, we have attempted to investigate some of the basic aspects of its chemistry and physiology.

From our data, some of which has been presented here, as well as an appraisal of the data of others, we believe the ultimate solution to control of fibrinolytic activity lies not in the use of exogenous enzymes but in stimulating the organism to produce its own activators and enzymes. It does not seem unreasonable to propose, as has von Kaulla,15 that this may be accomplished through the appropriate use of hitherto unrecognized pharmacologic agents. The effect in man of parenterally administered nicotinic acid30,31 is of interest in this connection. We are currently testing several compounds structurally related to nicotinic acid for similar activity. The possible neurologic control of the fibrinolytic enzyme system as suggested by the experiments of Kwaan et al.32 also provides an avenue of inquiry into this area.

A micromolecular approach to the inhibition of the plasma fibrinolytic enzyme system is provided by several substances. The chemical currently offering the greatest promise is the antiactivator, epsilon-aminocaproic acid. One can look into a future in which control of the fibrinolytic enzyme system will be as acceptable as control of prothrombin levels (by Dicumarol® and similar compounds) and of coagulation times (by heparin and related compounds).

SUMMARY

Development of methods for isolating, purifying and assaying urokinase, the urinary

enzyme activator of plasma profibrinolysin, is described. Properties of the enzyme are given and the unit of activity is defined and related to units of other workers. Studies of urokinase excretion are reported and note made of limitations which must be imposed on interpretation of variations in amount of enzyme excreted. Of particular importance is the possible existence of more than one molecular species of urokinase.

Plasma profibrinolysin preparations obtained from representatives of species ranging from man through lower mammals to fish can be activated by human urokinase. Profibrinolysin preparations from pig plasma are susceptible to urokinase activation only following removal of inhibitors.

Human urokinase has been used as a major tool in the detection of profibrinolysin in a variety of plasma protein preparations and in the development of procedures designed to remove profibrinolysin from such preparations as fibrinogen and thrombin. Human urokinase injected into heterologous species has been shown to be an antigen. This property has been utilized to demonstrate that parenterally administered human urokinase is excreted in the urine of injected rabbits with no apparent alteration in either enzymatic or immunologic characteristics.

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Pyrogens as Thrombolytic Agents

Clinical and Experimental Studies*

ERWIN DEUTSCH, M.D. and PETER ELSNER, M.D.

Vienna, Austria

BACTERIAL pyrogens exert many actions on the human organism. We were especially interested in four of them: (1) their actions on leukocytes; (2) fibrinolysis; (3) blood coagulation; and (4) body temperature. These effects have a certain time corelationship to each other which, however, does not necessarily imply a causal interrelationship (Fig. 1).

Leukopenia: The first effect of the intravenous injection of a bacterial pyrogen† is the development of a leukopenia. The number of leukocytes is reduced to 50 per cent or less at the end of the first hour. The leukopenia is caused by displacement of the leukocytes into the capillaries of certain organs such as lung, liver and spleen, and by disruption. Many enzymes and other leukocyte materials are liberated by this event. After 120 minutes the number of leukocytes begins to increase, and in the following hours a leukocytosis up to 20,000 cells and more may develop. This effect is so regular in normal persons that it may be used as a test for the reactivity of the bone marrow.

Fibrinolysis and Hypercoagulability: The next main effect is the development of fibrinolysis. It begins about sixty minutes after the injection, has its maximal activity after ninety minutes and has usually disappeared three hours after the injection. The fibrinolysis is preceded by a state of hypercoagulability, most clearly demonstrated with the thromboelastograph, and is characterized by a reduction of the reaction time.

Fever: The last effect is the increase of body temperature which begins with chills and shivering about ninety minutes after the injection and lasts for several hours.

† Pyrexal® (Wander), a lipopolysaccharide from Salmonella abortus equi.

FIBRINOLYTIC MECHANISM

We have been especially interested in the study of the mechanism of induction of fibrinolysis. A few remarks on methods may be permitted.

Methods of Study: We used the fibrin plate method of Astrup3 and Lassen.4 The results were checked with a casein digestion method, with the observation of the dissolution of the clot formed in the patient's diluted plasma and with the thromboelastogram. We used a system of heated fibrin plates with different additions, because it is practically impossible to compare the size of the lysed areas on standard and heated fibrin plates. Plasmin was determined without any addition; plasminogen with added streptokinase; activator with added partially purified bovine plasminogen; proactivator with added bovine plasminogen and streptokinase; and fibrinolysokinase with added human proactivator (milk) and bovine plasminogen. Redissolved plasma euglobulins were used. The heating time of the fibrin plates was extended to ninety minutes at 90°c. to be sure that the total amount of plasminogen was destroyed.5

Fibrinolytic Activator: A distinct fibrinolytic activity could be demonstrated on heated fibrin plates with redissolved plasma euglobulin showing the presence of free plasmin (Fig. 2). This free plasmin was not entirely formed during the long incubation period necessary for the fibrin plate method.6 This was demonstrated by the proteolytic activity against casein (Fig. 1). The high proteolytic activity on standard fibrin plates and heated fibrin plates after addition of partially purified bovine plasminogen demonstrates the presence of large amounts of an activator. The increase of the lysed area after addition of streptokinase proved that not all the plasminogen had been transformed to plasmin. This increase was smaller in patients with a high plasmin activity, and greater in patients with a

^{*} From the Central Coagulation Laboratory, the First Medical Department, University of Vienna School of Medicine, Vienna, Austria. This work was supported by grants from the Blood Research Foundation, Washington, D. C. and Dr. A. Wander A.G., Bern, Switzerland.

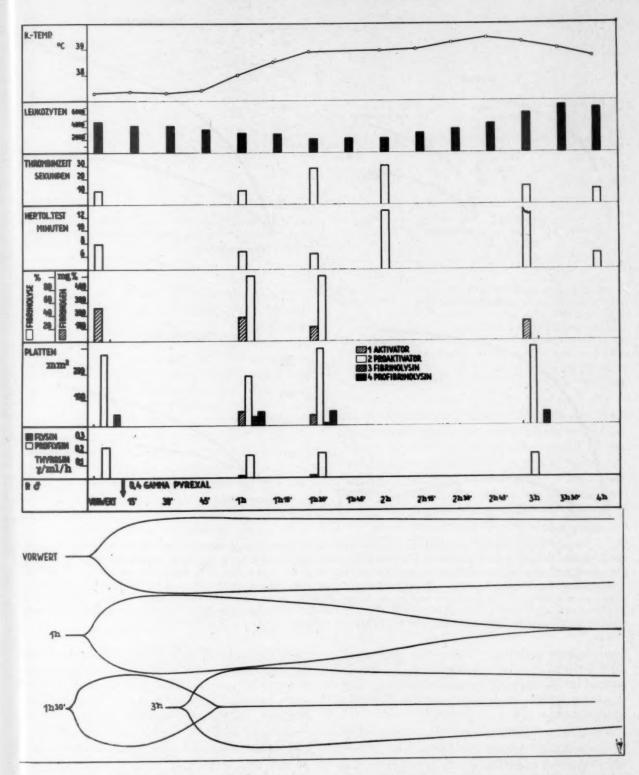


Fig. 1. The effect of 0.48 Pyrexal. First line: body temperature, in degrees Centigrade. Second line: leukocytes. Third line: thrombin times in seconds. Fourth line: heparin tolerance test in minutes. Fifth line: shaded column: fibrinogen in mg. per cent; unshaded columns: fibrinolysis in per cent of formed fibrin (lysis of the clot formed in diluted patient's plasma). Sixth line: fibrin plate method. All values in square mm. 1, shaded columns: free activator; 2, unshaded columns: proactivator (+ activator). 3, cross shaded columns: free plasmin; 4, black columns: plasminogen (+ plasmin). Seventh line: casein digestion method. γ tyrosine per ml. test solution per hour; black columns: free plasmin; unshaded columns: plasminogen + plasmin. Time in minutes after injection of pyrogen. At the bottom: thrombelastograms.

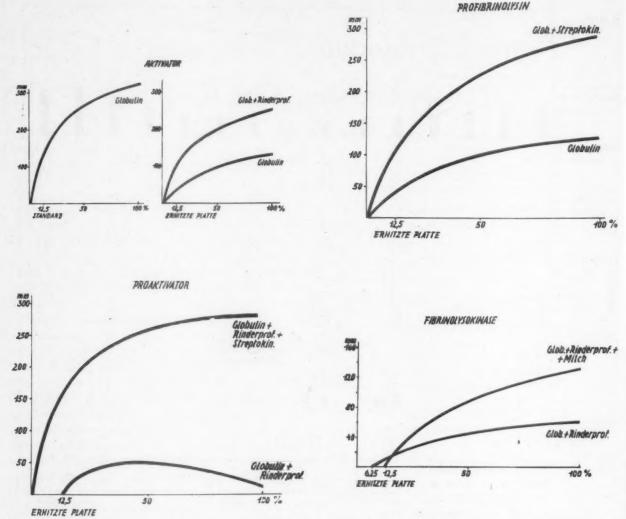


Fig. 2. Upper left, activator: comparison of euglobulins on standard plates, and lysed area by euglobulins on heated fibrin plates compared with euglobulins and bovine plasminogen added. Upper right, plasminogen: heated fibrin plates. Lysed area by euglobulins compared with that lysed by euglobulins with streptokinase added. Lower left, proactivator: heated fibrin plates. Activity of euglobulins with bovine plasminogen added compared with that of euglobulins with bovine plasminogen and streptokinase added. Lower right, fibrinolysokinase. Heated fibrin plates. Euglobulins with bovine plasminogen added compared with euglobulins with bovine plasminogen and proactivator (human milk) added. Abscissa—dilution of euglobulins. Ordinate—lysed area in square mm.

low plasmin activity. There was also proactivator left in the plasma which could be demonstrated by the increase of proteolysis after addition of bovine plasminogen and streptokinase in excess (Fig. 2).

These experiments suggest that the presence of an activator causes the fibrinolytic activity after injection of pyrogens. The addition of an excess of human proactivator resulted in an increase of the lysed area in only a few cases. This would suggest the presence of a fibrinolysokinase (Fig. 2).

Properties of Activator: A study of the properties of the activator could possibly give some information concerning its origin. It is known from the work of Astrup⁷ and Müllertz^{8,9} that the activator formed in plasma by streptokinase is labile at acid reaction, and even at neutral reaction it is destroyed at 50°c. The activator in tissues, on the other hand, is rather stable, especially at acid pH. We tested the stability of the activator formed after injection of pyrogens at different pH's and temperatures using the method of Astrup⁷ and Müllertz^{8,9} (Fig. 3). The activator was stable at acid pH at 70°c. and at 100°c. and was destroyed at neutral pH in the same temperature range. After incubation at 50°c. the activity was the same in the total pH range tested. Our activator corresponded in these properties to Astrup's stable

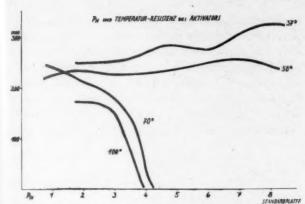


Fig. 3. The effect of incubation at different pH's and different temperatures on the activity of the activator. Standard fibrin plates. Abscissa-pH. Ordinate-lysed area in square mm.

type activator. However, if incubated at 37°C. the remaining activity at neutral pH was higher than at acid pH in a few cases. This finding suggests that a small amount of a labile type activator, blood activator, may be present in addition to the stable type activator, which possibly may originate from cells. But what is the source of the stable type activator?

It is well known that the extrinsic bacterial pyrogen does not induce fever by itself. During the period of lag between injection of the pyrogen and onset of the rise in temperature the extrinsic pyrogen is transformed into an intrinsic one. Leukocytes are obviously involved in this transformation. Cranston and co-workers 10 succeeded in producing an intrinsic pyrogen in vitro by incubation of the extrinsic pyrogen with blood or plasma containing leukocytes. Cell-

TABLE I Development of Fibrinolytic Activity

	Lysed Area (sq. mm.)						
Incubation Time, (hr.)	Citrate	ed Blood	Serum				
	Heated Plate	Standard Plate	Heated Plate	Standard Plate			
0.0	0	0	0	0			
0.5	0		0				
1.0	0		0				
1.5	0		0				
2.0	68	130	0	0			
2.5	58		0				
3.0	36		0	0			
3.5	33		. 0				
4.0	32		0				

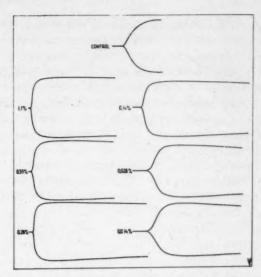


Fig. 4. Action of neutralized solutions of trypsin on the coagulation of platelet-rich plasma in the thrombelastograph. Two-tenths milliliter of plasma and 0.1 ml. of physiologic saline (control) or neutralized trypsin solution of different concentrations were clotted in the cup of the thromboelastograph by the addition of 0.06 ml. of 1.1 per cent CaCl₂ solution. The concentrations stated in the graph are final concentrations in the clotting mixture.

free serum was ineffective. We repeated these experiments and tested for fibrinolytic activity (Table 1). Fibrinolytic activity could be detected after an incubation period of two hours when citrated blood was incubated with a high dose of Pyrexal. No activity could be induced by incubation with serum. The importance of cleavage of leukocytes for the development of fibrinolytic activity is stressed by the observation of Brittingham who injected the blood of a person with leukoagglutins into a normal person, and induced leukopenia and a severe fibrinolytic reaction. The observation of spontaneous fibrinolysis in patients with myeloid leukemia may speak in the same sense. 12,18

EFFECT OF PYROGEN ON BLOOD COAGULATION

Hypercoagulability: Coagulation is influenced in two opposite ways. The predominant effect is the induction of a hypercoagulability which begins before fibrinolysis is fully developed, and may last for many hours. It is characterized by a shortening of the clotting time, the heparin tolerance test, and especially by a shortening of the reaction time in the thromboelastogram. This hypercoagulation effect is not a direct action of the pyrogen. The increase of coagulability is apparently a general side reaction of proteolytic enzymes, even in concentrations too low to induce effective fibrin-

olysis. We observed the same effect with nicotinic acid, and with streptokinase before fibrinolysis was induced, but pyrogens were the most active substances in this respect. We could also demonstrate this effect with an *in vitro* experiment with neutralized solutions of trypsin which did not induce proteolysis but shortened the reaction time of the thromboelastogram (Fig. 4).

Hypocoagulability: The opposite action on blood coagulation is caused by the action of plasmin, and is only evident when active fibrinolysis has developed. There is a reduction of factors v and viii and to a smaller degree of prothrombin and factor vii caused by proteolytic cleavage of these factors. The development of an antithrombin activity apparently is exerted

by fibrinogen degradation products.

ACTION OF BACTERIAL PYROGENS

We may summarize and correlate the actions of bacterial pyrogens as follows: The first action is the development of a leukopenia. At the same time a stable type activator of plasminogen is produced and the extrinsic pyrogen is transformed into the intrinsic pyrogen which finally causes the rise of body temperature. In vitro experiments suggest that the leukocytes are involved in both reactions. The activator transforms plasminogen into plasmin. In addition to fibrinolysis, the plasmin induces a hypercoagulability, and splits some clotting factors and fibrinogen. The split products of fibrinogen exert an antithrombin effect.

Meneghini¹⁴ and Stamm¹⁵ reported that swelling and edema disappeared faster in patients with venous thrombosis, and that ischemia was improved in shorter periods of time in patients with arterial occlusions when pyrogens were used. Nevertheless, a prompt repatency of the occluded vessels was observed only in a few cases. Repatency after a few weeks is, according to our views, recanalization which has noth-

ing to do with primary fibrinolysis.

We decided not to apply the pyrogen therapy to a great number of patients for the following reasons: (1) the fibrinolytic effect is too short-lived; (2) it is not possible to maintain fibrinolysis with pyrogens; (3) the dosage has to be high if fibrinolysis is to be induced regularly; (4) the method cannot be repeatedly applied to the same patient because the mechanism is soon exhausted; (5) the accompanying hypercoagulability is very undesirable. Therefore, it is necessary to combine this therapy from the very

beginning with anticoagulants; (6) the temperature rise is very inconvenient. It may, however, be suppressed by the simultaneous use of antipyretics; and (7) the contraindications are numerous: cardiac and circulatory insufficiency; marked hepatic and renal damage; adrenal insufficiency; severe diabetes; hemorrhagic conditions; hypertension; and old age.

SUMMARY

Four effects of intravenously injected purified bacterial pyrogens are discussed:

1. The induction of a leukopenia within the first hour after injection of the pyrogen. This is followed by a leukocytosis lasting for many hours.

2. The induction of fibrinolysis beginning about sixty minutes after the injection and lasting for about ninety minutes. It is caused by the appearance of a great amount of a stable type activator in addition to a small amount of a labile type activator. In vitro experiments and some clinical observations suggest that the leukocytes may be a source of the stable type activator which may be liberated from the leukocytes (and possibly other cells) at the time of development of leukopenia. The activator induces the formation of free plasmin. We found in only a few cases, that another mechanism was responsible for the activation of fibrinolysis, a lysokinase, which induces the formation of a labile type activator.

3. Effects on blood coagulation are not caused by the pyrogen itself. The most obvious effect is the development of a hypercoagulability, which could be demonstrated to be a general side effect of the action of proteolytic enzymes in low concentrations. The same effect, but less marked, may be observed after administration of nicotinic acid, small amounts of streptokinase, and trypsin. When the proteolytic activity is fully developed, a digestion of factors v and v III and to a smaller degree of prothrombin and factor v II is observed. Splitting products of fibrinogen cause a distinct antithrombin activity.

4. The body temperature is elevated. A period of about ninety minutes after the injection is necessary for the formation of the intrinsic pyrogen which finally causes the rise in temperature. Leukocytes are apparently involved in this transformation.

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- DISCUSSION OF PAPERS BY DRS. SGOURIS, IN-MAN AND McCall; Celander and Guest; AND DEUTSCH AND ELSNER

DR. WEINER: One word of caution about equating a shortened R value on the thrombelastograph with hypercoagulability as we see it in the test tube: the beginning of motion on the thrombelastograph represents not really the time that coagulation begins to take place, but the time that the clot becomes firm enough to move the apparatus. In our studies on nicotinic acid, for instance, we have seen (as Dr. Deutsch reported) some shortening of the R value, but frequently in the test tube we cannot see a shortening of the clotting time simultaneously. Our interpretation is that the firmness of the clot was developing somewhat more rapidly, but not necessarily that the visible clot has speeded up in its development.

It is interesting that both the pyrogen and the nicotinic acid mechanism of activating lysis seem to be self-exhausting. Apparently they have some common lysis-initiating mechanism.

DR. JOHN S. LADUE (New York, New York): Have you made any efforts to obtain activator from leukocyte concentrates?

DR. ERWIN DEUTSCH (Vienna, Austria): When we compare clotting tests with R times in the thromboelastograms, we always find a sharp corelationship between the two. The firmness of the clot is characterized by the size of the excursions and not by the R time. When we made heparin tolerance tests at the same time, we found parallel shortening of the heparin tolerance tests. Therefore, I think we have to deal with the clotting time as well as the firmness of the clot. The platelets behaved differently from one patient to the next. It is not the same with the leukocytes, which regularly decrease and form leukopenia. In some cases we found a thrombopenia; in some cases we found thrombocytosis. As to the last question, we did not make any efforts to purify this activator from the leukocytes.

Factors Affecting the Formation and Dissolution of Experimental Thrombi*

ALVIN H. FREIMAN, M.D., NILS U. BANG, M.D., CARLO E. GROSSI, M.D. and EUGENE E. CLIFFTON, M.D.

New York, New York

Recent interest in the use of fibrinolytic agents for the treatment of human thromboembolism¹⁻⁸ has emphasized the need for investigation of the factors responsible for clot formation and dissolution. The experiments to be described were undertaken to demonstrate some of the factors which might determine the response to fibrinolytic therapy. It was hoped that this experimental data would result in a better understanding of the usefulness and limitations of fibrinolytic agents in clinical situations.

The first requirement for such a study was to establish a uniform type of clot which could then be altered to suit the particular experiment. Such a clot should be composed of the animal's own blood and should preferably not contain contaminants such as bovine thrombin or bovine fibrinogen which have previously been shown to contain high concentrations of profibrinolysin.⁴ It was also desirable to be able to demonstrate such a clot and follow progress objectively.

We used the method originally described by Wessler⁵ for forming clots. In this technic, the injection of homologous serum at a distant site is followed by clamping of the vessel to be clotted. This results in the formation of a firm, non-adherent clot at the desired site, a clot which grossly and histologically bears a close relationship to those seen in human phlebothrombosis. In some circumstances, clots were formed by the use of sodium morrhuate for comparative purposes. The formation of the latter type of clot involves significant endothelial damage and is more analogous to that seen in inflammatory thrombophlebitis.

MATERIALS AND METHODS

Mongrel dogs ranging in weight from 6.5 to 18 kg. were anesthetized with pentobarbital, 25 mg. per

kg. administered intravenously. Serum for clot formation was produced by allowing clotted blood from a donor dog to remain at room temperature for thirty minutes, following which it was centrifuged at 3,000 r.p.m. for fifteen minutes at 10°c. In the initial experiments, the original Wessler technic was used, but later a modification of this technic was devised in which a 3 cm. segment of vessel was isolated between clamps, following which 0.15 ml. of serum was injected into the vessel through a No. 30 needle. By this technic bulging clots were consistently produced. A stenosing ligature producing a 60 to 75 per cent occlusion of the vessel lumen was placed prior to removal of the clamps to prevent escape of the clot, and a radiopaque marker was attached at this point to delineate the distal end of the clot. In some cases sodium morrhuate clots were produced by a previously described method.6 In all cases a proximal side branch of the vessel containing a clot was catheterized with fine polyethylene tubing to permit angiography and direct infusion above the clot.

Three groups of experiments were carried out as follows: *Group I:* the effect of fibrinolytic treatment on freshly formed clots; *Group II:* the effect of fibrinolytic treatment on clots of varying ages; and *Group III:* the effect of fibrinolytic therapy on clots of varying fibrinogen content.

Group I: In this group the effectiveness of treatment with systemic fibrinolysin† was compared with the effectiveness of fibrinolysin administered directly through the catheter in close relation to the clot. For this purpose, clots were formed in two vessels at the same time and treatment with fibrinolysin was instituted through the catheter in the side branch on one side. This, in effect, served to treat one clot locally, while the other clot was affected only by the systemic circulating fibrinolysin. Four thousand to 8,000 Christensen units per kg. per hour were administered with a resulting euglobulin fibrinolytic activity

† Fibrinolysin (SK activated plasminogen), Merck Sharp & Dohme, Lot No. 1108-86N, supplied through the courtesy of Werner Baumgarten, Ph.D., West Point, Pennsylvania.

^{*} From the Clotting Mechanisms Section, Division of Experimental Surgery and Physiology, Sloan-Kettering Institute for Cancer Research, and the Department of Surgery, Cornell University Medical College, New York, New York. This study was supported in part by Grant H-2867 from the National Institutes of Health.

Table 1
Time Required for Complete Lysis of Fresh Clots

Location of Clot and Type of Therapy	No. of Cases	Average Time	Range (hr.)	
Venous clots Local adminis-				
tration	4	2 hr., 15 min.	1.5-3	
Systemic ad- ministration Arterial clots	5	4 hr., 10 min.	2.5-5.5	
Systemic ad- ministration	4	4 hr.	3-5	

in the range of ten to twenty-five minutes. Serial angiograms with 4 ml. of radiopaque dye* were performed after clot formation and at intervals of one to two hours during therapy. When the x-ray studies demonstrated complete lysis, the experiment was stopped. Control animals received 5 per cent glucose in water through the catheter.

Group II: One serum and one morrhuate clot were formed in each animal on respective sides. At periods of time after formation from six hours to three weeks, the animals were reanesthetized, the vessels were exposed and the side branches catheterized. Then 42,000 units of fibrinolysin per hour for six hours was given through a distant femoral vein. At the end of this time, the vessels were removed and after suitable fixation in formalin were sectioned and stained with phosphotungstic acid, Masson's tri-chrome and hematoxylin and eosin. The results of treatment were evaluated by both x-ray and autopsy findings, as (1) No lysis: By this was meant that there was still complete obstruction to passage of the dve and a large clot was still found; (2) Partial lysis: In this case, dye could pass below the stenosing ligature, filling defects were still apparent and small amounts of clot were found; (3) Complete lysis: Here no filling defect was demonstrable and no clot was found after removal of the vessel.

Group III: In this group a clot was formed by the use of serum on one side, a side branch was catheterized and the clot was demonstrated by an angiogram. Then 10 gm. of bovine fibrinogen dissolved in 100 ml. of isotonic saline was given intravenously. Immediately after, the contralateral vessel was clamped and a thrombus was formed on the other side by the use of serum. As a result a clot with normal fibrinogen content was formed first, while the second clot was relatively higher in fibrinogen. After the second clot was demonstrated by angiography, 42,000 units of fibrinolysin per hour for six hours was administered through a distal vessel. Blood samples were taken

TABLE II

Degree of Lysis Obtained with Fibrinolysin Therapy of Fresh Clots as Evaluated by Angiograms and Autopsy

Location of Clot and Type of Therapy	No. of Cases	Com- plete	Partial	No Lysis
Arterial clots Systemic				
therapy Venous clots	6	4	2	0
Local therapy Systemic	4	4	-0	0
therapy	14	8	5	1

before the administration of fibrinogen, after the fibrinogen had been infused and after three hours of therapy with fibrinolysin. These samples were tested for fibrinogen concentration, fibrinolytic activity, antiplasmin fibrinolytic activity and whole clot lysis. The sections of vessel were removed after the six-hour period of therapy and were sectioned and stained as before. The results of therapy were evaluated by x-ray and autopsy studies according to the criteria of no lysis, partial lysis and complete lysis as described.

RESULTS

Group I: Sixteen dogs were treated with fibrinolysin after fresh clots had been formed. Three dogs served as controls receiving 5 per cent glucose in water and in all three the clots remained stable during the course of the experiment. Six animals were treated with systemic therapy while in two other dogs local therapy alone was used. In two additional animals, thrombi were formed on both sides and one clot was treated locally while the other was affected only by systemic circulating fibrinolysin. The average time required for lysis of clots treated systemically was four hours and forty-five minutes, while the average time required for complete lysis by local therapy was two hours and ten minutes (Table 1).

In five dogs the relative effectiveness of systemic therapy of arterial and venous clots was tested. These clots were formed simultaneously in each animal. There was no significant difference in the susceptibility of venous and arterial clots to fibrinolysin, when treated systemically (Table II).

The doses of fibrinolysin used resulted in euglobulin fibrinolytic activity in the range of eight to twenty-five minutes with an average of fourteen minutes. This level of activity was noted within thirty minutes to two hours after the start of therapy.

^{*} Sodium diatrizoate, 50 per cent, available as Hypaque® from Winthrop Laboratories, New York, New York.

TABLE III

Results of Fibinolysin Therapy of Clots of Varying Ages

Time Degree of Lysis Between Clot No. of Formation Animals Morrhuate Serum and Clots Clots Treatment Complete 6 hr. 1 Complete 24 hr. 1 Partial Complete Complete Complete 48 hr. 2 2 Partial Complete 3 days 4 days 2 Partial Partial 2 5 days None None 7 days 1 None None None 9 days None 1 16 days None None 3 wk. None None 1

It was of interest to note that in two dogs who had received an overdose of pentobarbital resulting in episodes of shock and respiratory arrest, strong fibrinolytic and proteolytic activity was found during, and persisted for ten to twelve hours after, the episode. Attempts to form clots in these animals by the use of serum or thrombi were completely unsuccessful.

Group II: Twenty-one dogs were used. Clots were formed aseptically following which treatment was established at the times noted previously. Clots were demonstrated in all cases by angiograms prior to therapy. It was found (Table III) that there was evidence of lysis of all clots treated within four days of formation. All serum clots up to forty-eight hours of age were lysed completely, whereas only partial lysis was noted in some of the morrhuate clots treated within this period of time. Serum clots treated three and four days after formation showed only partial lysis. No clear-cut evidence of lysis could be demonstrated on clots older than four days. Microscopic examination of the clots did not disclose any significant difference between those that were less than four days old and those that were five or six days old. In particular, the development of resistance to dissolution by fibrinolysin did not correlate with the appearance of organization or endothelialization. However, the gross appearance of the clot changed within twenty-four hours in that it lost its jelly-like consistency and became increasingly firm.

Group III: Thirteen dogs were included in this study. Three animals died in anaphylactic

Table IV

Results of Fibrinolysin Therapy of Clots of Varying
Fibrinogen Contents

Dog No.	Low Fibrino- gen Concen- tration (mg./100 cc.)	High Fibrino- gen Concen- tration (mg./100 cc.)	Low Fibrino- gen Clot Lysed (hr.)	High Fibrino- gen Clot Lysed (hr.)
29	282	440	3.5	5.5
30	250	400	4.5	6.0
53	347	472	4.5	Partial
54	352	450	4.0	Partial
55	284	470	2.5	Partial
56	246	380	3.5	Partial
57	300	472	3.75	Partial
58	334	450	5.0	Partial
59	269	418	5.0	Partial
60	278	350	4.5	Partial

shock immediately after the rapid administration of bovine fibrinolysin. In the remaining ten dogs the circulating fibrinogenic level increased from 15 to 60 per cent after infusion of fibrinogen. The dose of fibrinolysin used resulted in euglobulin fibrinolytic activity ranging from ten to forty-five minutes in all animals. This was maintained during the entire standard six hours of treatment used in this series of experiments. While there was no exact relationship between the absolute fibrinogen concentration and the rate of clot dissolution, the clots formed during the hyperfibrinogenemic states were significantly more resistant to lysis than those formed when the fibrinogen concentration was normal (Table IV).

COMMENTS

The serum-induced clot, as described by Wessler or as we have modified it, appears to be a suitable standard clot for the evaluation of factors which may affect the results of fibrinolytic therapy. It has the advantage of not requiring the use of substrate or additives foreign to the experimental animal. In addition it has appeared to be the closest experimental approximation to human phlebothrombosis.

In these experimental procedures we have not been concerned with the effect of dosage levels or duration of therapy upon the degree of lysis of clots. We have purposely administered fibrinolysin in extremely high doses to enable us to evaluate other factors.

The more rapid and complete lysis of clots

when treated locally as against systemic therapy emphasizes the importance of continuous delivery to the clot of a high concentration of enzyme or activator. Systemic therapy, although effective, is probably less so because of the presence of circulating inhibitors.

The finding that fibrinolysin in high doses was equally effective against arterial and venous clots is somewhat different from that of other authors who noted that higher doses were required for dissolution of arterial clots. In these experiments collateral circulation to the veins was cut off and it is probable that such collateral circulation is important in providing increased delivery of enzyme to the thrombosed area.

The observation that persistent clots could not be formed in the fibrinolytic state has been of interest to us from two points. The intense fibrinolytic activity demonstrated points up the fibrinolytic potential which the body possesses and raises a question of the possible application of maintained fibrinolysis in a prophylatic sense.

Effect of Age of Clot: The result of treatment of clots of increasing age confirms previous studies of the time limits for successful therapy.^{2,7} We have not, however, been able to substantiate the finding that appearance of resistance to lysis is associated with the organization of the clot and its overgrowth with vascular endothelium. The grossly increased clot density may, however, affect the diffusion of enzyme or activator into the clot, or some more subtle change in the fibrin polymer at the molecular level may affect activator of intrinsic profibrinolysin within the clot. The effect of the age of a clinical thrombus on its response to therapy arises in this study. It is important to note that human clots represent in all probability a dynamic process with continued recent extensions of thrombus. Thus, while the original clot may not be amenable to therapy, its more recent additives may be susceptible to successful treatment. If these recent additives are affecting the collateral circulation, considerable clinical benefit might result from therapy with fibrinolysin.

Hyperfibrinogenemia: The effect of hyperfibrinogenemia on the type of clot formed and its response to fibrinolysin may have its clinical counterpart. Hyperfibrinogenemia can be observed after coronary thrombosis, postoperatively and in many inflammatory diseases, a setting in which thromboembolism is frequently seen. If, as has been postulated, fibrinolysis represents a bodily defense against formation and extension of such thrombi, it is conceivable that such a defense mechanism may be considerably less effective against clots formed during hyperfibrinogenemic states and that hyperfibrinogenemia may play a role as a thrombogenic factor.

SUMMARY

The use of the serum-induced clot as a standard experimental model has been found to be highly satisfactory.

In twenty-two dogs the effectiveness of fibrinolytic therapy of fresh clots was studied. Systemic fibrinolysin therapy was found to be effective in dissolving such clots, although local administration of fibrinolysin to the clot was found to result in even more rapid lysis. No difference was found in the susceptibility of fresh arterial and venous clots to treatment.

In twenty-one dogs the effect of the age of the clot in relation to treatment with fibrinolysin was studied. Clots treated within four days of formation were susceptible to partial or total dissolution.

The effect of hyperfibrinogenemia on clot dissolution was studied in ten dogs. Clots formed in the hyperfibrinogenemic state were significantly more resistant to fibrinolytic therapy.

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DISCUSSION OF PAPER BY DRS. FREIMAN, BANG, GROSSI AND CLIFFTON

DR. H. OUCHI (Boston, Massachusetts): I would like to limit my discussion to the following two

subjects: (1) experimental induction of intravascular thrombi in dogs and (2) their dissolution with human fibrinolysins. Our primary concern in the experimental induction of intravascular thrombi was to obtain "standard" thrombi in terms of size, age and character for the purpose of comparison of therapeutic effects. Two partial constrictions were made in the jugular vein in a dog. A partially constricting bulldog clamp with a hole in the middle was applied and exactly 4 cm. distal to the clamp, a partial constriction was made with a cotton suture. After fifteen minutes, 2 ml. of stagnating venous blood was slowly taken into a syringe. Without removing the needle, 1 ml. of the 2 ml. was rapidly injected into the partially constricted vein segment. A firm and non-adherent clot was formed. Twelve clots thus formed were removed from the vein for measurement of their size and observation of character. They were reasonably uniform in size and had the appearance of postmortem intravascular thrombi. A control group of clots was studied, varying in age from fresh to seven days. A saline solution given locally to fresh thrombi for a period of one hour did not produce any significant change of their size. The control group was, therefore, established by removing them for measurement of their volume. Intravascular clot retraction produced a volume decrease of 33 per cent within a seven-day period. The amount of thrombolysis from local administration of plasmin was also studied. When infusion was for a one-hour period, only an extremely high concentration of plasmin could induce effective thrombolysis. We have also performed systemic administration of plasmin. I cannot discuss this further except to say that comparatively high doses were necessary to produce thrombolysis when this method was employed.

DR. STANFORD WESSLER (Boston Massachusetts): Dr. David G. Freiman and I at the Beth Israel Hospital in Boston have been interested in studying the natural life history of thromboembolic phenomena with the technic of serum-induced thrombosis mentioned by Dr. Alvin Freiman. To our knowledge this is the only available experimental technic for producing thrombosis that is known to operate via the so-called intrinsic clotting system. The induction of thrombosis is therefore independent of tissue thromboplastin. The method is simple and reproducible. The thrombi formed are of uniform composition, can be of predetermined size and can be formed without demonstrable intimal damage or significant systemic disturbance in arteries and veins of a variety of animals. Subsequent to formation these thrombi can be released to vascular beds such as the pulmonary artery or portal venous systems. Finally, the histologic counterpart of these thrombi has been observed in man. Employing this technic in the untreated animal, we have studied the response of the dog's lytic mechanism to three types of pulmonary emboli: (1) single, small, fresh emboli 2 to 14 cm. in length; (2) massive, fresh emboli 20 to 90 cm. in length; and (3) single, small, aged emboli 2 to 4

cm. in length. In each instance the embolus represented a thrombus of the animal's own blood formed in a peripheral vein by the systemic infusion of serum. In twenty dogs sacrificed four hours after thrombus release, emboli were recovered from the right side of the heart or pulmonary arteries in each animal. Some emboli reached the pulmonary artery intact, others were trapped in the right heart by the chordae tendinae of the tricuspid valve and some underwent fracture. In six dogs examined one to four days after release of thrombi, emboli were also recovered in every animal but only one was found in the right side of the heart. From the fifth to the thirteenth day emboli were recovered in approximately half the animals and in five dogs permitted to survive fifteen to twenty-eight days no emboli could be recovered from the right side of the heart or the pulmonary arterial tree. In contrast to the small, fresh emboli, many centimeters of thrombi could be released to the lung partly filling most of the pulmonary arterial branches. Such emboli were found not only in every animal sacrificed up to the sixteenth day but also in four of six animals sacrificed from the forty-third day to the sixth month. After eight days, however, there was a tremendous reduction in the size of the embolus, and after one month only minute nubbins could be found on gross dissection. Finally, small thrombi 2 to 4 cm. in length were aged in a jugular vein for fourteen days before being released to the pulmonary artery. These emboli were recovered in almost every instance. They appeared to pass through the right side of the heart more rapidly than fresh emboli, rarely if ever underwent fracture and were recognizable as the original thrombus for weeks in the pulmonary artery. Only after several months were they reduced to small nubbins.

These investigations suggested to us the following interpretations: (1) It is apparent that there is an effective lytic mechanism which in a short period of time causes fresh emboli to disappear. (2) The capacity of this normal mechanism is enormous as shown by the dramatic reduction of massive fresh emboli in short periods of time. (3) When the quantity of thrombus is sufficiently great, however, the normal lytic mechanism may be overloaded, resulting in the persistence of emboli which go on in time to organization. (4) Alteration in substrate, here accomplished by aging, appears to slow the rate of thrombolysis. Even aged thrombi may be dissolved in time by the normal lytic mechanism. And, finally, we would like to suggest that the technic of serum-induced thrombosis provides standardization of the target thrombus which should prove useful in the study of induced alterations in normal lytic activ-

DR. DAVID R. CELANDER (Galveston, Texas): I am curious about the three dogs with spontaneous fibrinolytic activity. Could you give us a few comments on their background and the nature of the anesthetic employed?

DR. ALVIN FREIMAN (New York, New York): The

anesthetic was veterinary Nembutal. It was given in apparently slightly more than the usual dose and the dog was noted to be slightly blue and was hypoxic for several minutes before we could begin artificial respiration. Then the animal appeared all right and we carried on the experiment except that we were unable to form a clot. We found euglobulin fibrinolytic activity in the second and third range of about thirty minutes.

DR. N. BACK (Buffalo, New York): In 1958, in the Journal of Clinical Investigation, we reported on the fibrinolytic effect of plasmin administered intravenously to dogs carrying clots in various stages of organization. We showed, as you have, that older clots were less subject to lysis by plasmin. In dogs receiving only saline, standard half-hour old clots lysed approximately 23 per cent. These clots were formed with I¹³¹-labeled fibrinogen and clot lysis correlated well with disappearance of radioactivity. Following plasmin therapy, clots three days, five days and ten days of age lysed to the same extent as control clots. Clots half-hour, one day and two days old, however, showed considerable lysis, 80 to 97 per cent. These

results were obtained with 30 Loomis units of plasmin per kilogram body weight. Histopathologic study revealed the presence of an endothelial lining formed by the third day around the free edges of the clot. The lining possibly prevented intimate contact between the injected plasmin and the fibrin mass.

Dr. Freiman, what type of units did you use when you indicated 5,000 units/kg.? Did you see any endothelialization in any aged clot you studied?

DR. FREIMAN: In answer to the first question, we used Christensen units. In response to the second question, we saw significant resistance to lysis of these aging clots well before the appearance of significant endothelialization, in contradistinction to what you found and we wondered how much of this endothelialization was a reaction to actual vessel trauma rather than to the presence of a bland non-inflammatory clot. We tried to correlate what this increased resistance was due to but we do not have a satisfactory answer. On examining the clots they look a great deal different. They become much denser in nature after several days. It is possible that diffusion into these clots would be a more difficult physical process.



Experiences with Inhibitors to the Plasmin-Plasminogen System in the Human Subject

Their Modifications by Thrombolysin Therapy*

HERSCHEL SANDBERG, M.D., GEORGE TSITOURIS, M.D. and SAMUEL BELLET, M.D., F.A.C.C., WITH THE TECHNICAL ASSISTANCE OF JEAN SCHRAEDER, M.T. (ASCP)

Philadelphia, Pennsylvania

THE APPEARANCE of highly purified proteo-I lytic and fibrinolytic enzymes during the past decade1-8 has stimulated a growing interest in the mechanisms of fibrinolysis. Several investigators have suggested that impaired fibrinolytic mechanisms may be the basis for the pathogenesis of atherosclerosis.4-6 Our early experiences in trying to correlate the dosage of fibrinolytic enzyme administered to patients with thromboembolic disorders with the ability of their blood to dissolve fibrin clots in vitro, led us to suspect that increased amounts of antithrombolytic substances appeared in the plasma during these disease states. Methods were therefore devised to further investigate this problem.

MATERIAL AND METHODS

The subjects of this study consisted of three groups: (1) twenty-nine members of the resident staff and laboratory personnel of the Division of Cardiology of the Philadelphia General Hospital (control group); (2) fifty-eight patients with acute myocardial infarction (twenty-one received no heparin; the remainder had been treated with heparin); and (3) twenty-two subjects with other thromboembolic disorders (e.g., acute thrombophlebitis, pulmonary embolism, cerebral thrombosis). The plasma was obtained and prepared for analysis in a manner previously described. Plasma fibrinogen concentration was determined by the quantitative method of Stefanini and Dameshek.

The samples were tested for antithrombolytic activity in the following manner: An incubation mixture was prepared consisting of (1) 0.2 ml. of 1 per cent fibrinogen in imidazole-saline buffer (ISB); (2) 0.2 ml. of human thrombin in ISB (20)

units/ml.); (3) 0.2 ml. of test plasma; and (4) 0.4 ml. of thrombolysin.† The tubes were placed in a 37° c. water bath and the lysis times determined by inverting the tubes at regular intervals until the clot failed to cling to the bottom of the tube. The time required to lyse this fibrin clot was considered to be a measure of the antithrombolytic activity (inhibitor activity) of the test plasma.

RESULTS

Control Group: The antithrombolysin lysis times and fibrinogen concentrations of the normal control group have been previously reported. When 80 Merck units (1:10 dilution in ISB) were incubated with normal control plasma, the mean lysis time of the standard fibrin clot was 211 ± 47 seconds; with 40 Merck units (1:20 dilution in ISB) the mean lysis time was 468 ± 146 seconds; with 20 Merck units (1:40 dilution in ISB) the mean lysis time was 755 ± 191 seconds.

Myocardial Infarction: The antithrombolysin lysis times (against 40 Merck units of thrombolysin) of patients with acute myocardial infarction are represented by the height of the hatched columns in Figures 1 and 2. The solid columns on the left represent the values of normal control subjects. Each column represents an individual subject. In Figure 1 the patients with acute myocardial infarction had received no heparin; in Figure 2 they had received anticoagulant therapy with heparin. It is

† Merck, Sharpe & Dohme human fibrinolysin. Three dilutions in ISB are used: 1:10, 1:20 and 1:40 containing 80, 40 and 20 Merck units of thrombolysin, respectively.

^{*} From the Division of Cardiology, Philadelphia General Hospital, Philadelphia, Pennsylvania. This work was aided by a grant from the U. S. Public Health Service (H141-C8), and by Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania.

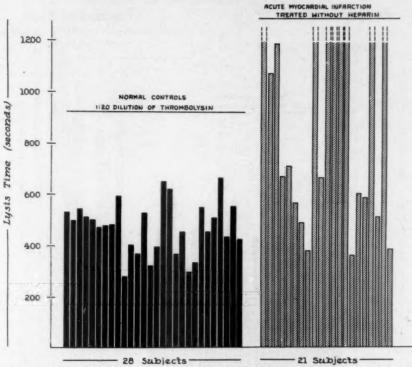


Fig. 1. Inhibition of fibrinolytic activity in normal subjects and in patients with myocardial infarction. The antithrombolysin lysis times against 40 Merck units of thrombolysin are shown by the height of the columns. The hatched columns on the right represent subjects with acute myocardial infarction who did not receive heparin; the solid columns on the left represent twenty-eight normal control subjects. The inhibition to fibrinolytic activity (as measured by the antithrombolysin lysis times) is considerably greater in patients with myocardial infarction than in the normal control subjects.

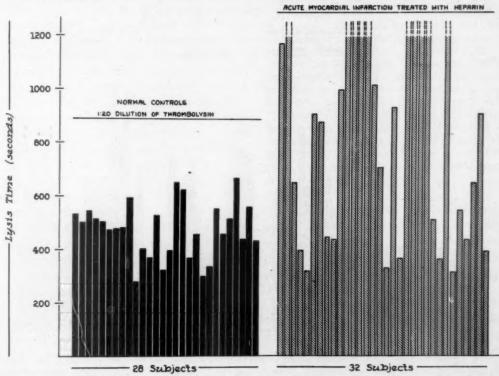


Fig. 2. The antithrombolysin lysis times of thirty-two patients with acute myocardial infarction, who had been treated with heparin, are shown by the hatched columns on the right. The solid columns on the left represent the normal control subjects. Again it is noted that inhibition to fibrinolytic activity is greater in the patients with myocardial infarction than in the normal control subjects.

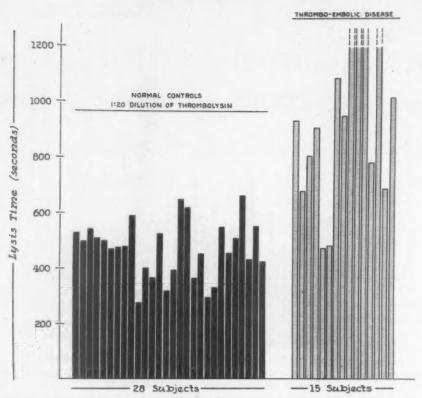


Fig. 3. The dotted columns on the right depict the antithrombolysin lysis times in fifteen patients with thromboembolic disease; the solid columns on the left represent the inhibition to fibrinolytic activity found in the normal control subjects. There is much greater inhibition to fibrinolytic activity in the patients with thromboembolic disease than in the normal control group.

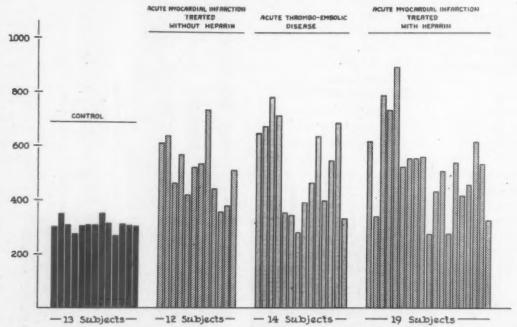


Fig. 4. Plasma fibrinogen concentrations. The solid columns on the left represent the plasma fibrinogen concentration (mg./100 ml.) of the normal control group. The remaining three columns, left to right, represent the plasma fibrinogen concentration of patients with acute myocardial infarction who had not received heparin, patients with acute thromboembolic disease, and patients with acute myocardial infarction who had been treated with heparin. The plasma fibrinogen concentrations in the latter three groups are considerably higher than those of the normal control group.

Table 1

Effects of Increasing Plasma Fibrinogen Concentration on the Antithrombolysin Time

Fibrin-	Antithrombolysin Time (sec.)					
ogen Concen- tration (mg./ml.)	Thrombo- lysin Diluted 1:10 in ISB	Thrombolysin Diluted 1:20 in ISB	Thrombo- lysin Diluted 1:40 in ISB			
10	190	316	575			
11	225	310	561			
12	267	303	611			
13	265	310	569			
14	245	354	557			
15	249	375	611			
16	260	368	626			
17	291	377	613			
18	260	369	665			
19	289	453	627			
20	282	457	609			

Note: Assay mixture consisted of the following: 0.2 ml. fibrinogen solution, 0.4 thrombolysin solution, 0.2 ml. thrombin and 0.2 ml. normal plasma (fibrinogen concentration 302 mg./100 ml.).

readily seen that the patients with acute myocardial infarction have considerably longer antithrombolysin lysis times than those of the control group. Moreover, there is no appreciable difference between the patients who had received heparin and those who did not.

Thromboembolic Diseases: Figure 3 illustrates similar studies in patients with thromboembolic diseases. In these patients, also, the times required for the mixture to dissolve a standard fibrin clot are considerably longer than those of the control group. The dotted columns on the right depict the antithrombolysin lysis times (against 40 Merck units of thrombolysin) of the subjects with thromboembolic disease. The solid columns on the left represent the normal control subjects.

Plasma Fibrinogen Concentrations: Figure 4 illustrates the plasma fibrinogen concentrations in patients with various thromboembolic disorders compared to those of the normal control subjects. These values in all the thromboembolic diseases are higher than those of the control group.

To rule out the possibility that these increased lysis times are merely artifacts due to the increase in plasma fibrinogen concentration which occurs in the aforementioned conditions, our assay system was modified in the following manner: Instead of utilizing 1 per cent fibrino-

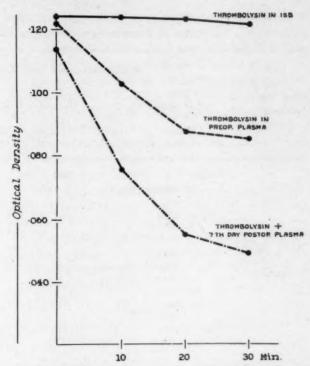


Fig. 5. Inhibition to thrombolysin activity by means of a proteolytic assay on casein, pre- and postoperatively. The solid upper line represents the optical density produced on casein substrates by thrombolysin incubated with ISB. There is little decrease in the optical density (and hence the proteolytic activity) between the thrombolysin which had been incubated ten minutes with ISB and that incubated thirty minutes with ISB prior to the final thirty-minute reaction with casein. middle broken line shows the optical density produced when the thrombolysin is incubated with preoperative plasminogen-free plasma. The progressive decrease in optical density as the incubation time is prolonged illustrates the inhibitory substances to thrombolysin proteolytic activity present in the preoperative plasma. The lower line illustrates the results of incubation of thrombolysin with the plasminogen-free plasma of the same patient drawn on the seventh postoperative day. Much greater inhibition to thrombolysin activity is present in the postoperative sample than in the preoperative one.

gen in the assay, serial concentrations of fibrinogen, ranging from 10 to 20 mg./ml., were added to a normal control plasma (fibrinogen concentration 302 mg./100 ml.) and the variation in the antithrombolysin lysis times were noted. The results are shown in Table 1. Although there is some prolongation of the time required to dissolve the fibrin clot, particularly in the higher concentrations of fibrinogen, it is not of the magnitude shown in the previous figures.

Inhibition of Proteolytic Activity: In a few instances the plasma inhibition to thrombolysin activity was also studied by casein proteolysis. The effect is well demonstrated in Figure 5,

TABLE II

The Effects of Thrombolysin Therapy on Parameters of the Fibrinolytic System in Man

	Time of Adminis-	Antithrombolysin Lysis Time (sec.)			Plasma Lysis Time (sec.)		Faralaka Ka	Plasma Fibrin-
	tration, Amount Given (Merck units)	Thrombo- lysin Diluted 1:10 in ISB	Thrombolysin Diluted 1:20 in ISB	Thrombo- lysin Diluted 1:40 in ISB	Undiluted Plasma	Plasma Diluted 1:20 in ISB	Euglobulin Lysis Time (sec.)	ogen Concen- tration (mg. %)
	None 1/2 hr. after, 200,000	302 No clot	614 No clot	733 140	1,152 184	1,200+ 608	1,200+	868 850
	12 hr. after, 200,000	No clot	No clot	170	186	712	630	860
J. T., M, 34,	24 hr. after, 200,000	84	113	466	209	934	643	854
pulmonary embolus 1/2 hr. after 2nd dose 200,000 3 hr. after	1/2 hr. after 2nd dose,	No clot	60	94	No clot	563	470	835
	3 hr. after 2nd dose	78	110	309	No clot	539	836	850
	24 hr. after 2nd dose	62	128 364 No clot 764 1	1,104	854			
300,0 18 hr. a 300,0 18 hr. a 300,0 36 hr. a 300,0 60 hr. a	2 hr. after,	369 · 137	699 168	1,200+ 374	1,200+ 277	1,200+	1,200+ 510	497 379
	18 hr. after,	146	230	527	694	1,200+	880	382
	36 hr. after,	172	411	628	1,054	1,200+	1,200+	
	60 hr. after, 300,000	293	456.	757	1,200+	1,200+	1,200+	
A. B., M. 54,	None 2 hr. after, 300,000	277 No clot	700 No clot	1,200+	1,200+	1,200+ 421	1,200+ 392	378
right super- ficial throm-	12 hr. after, 300,000	143	250	410	420	1,200+	1,200+	
bophlebitis	2 hr. after, 300,000	No clot	No clot	No clot	No clot	133	279	353

which illustrates proteolytic inhibition produced by major surgery. The solid upper line represents the optical density produced on casein substrates by thrombolysin incubated with ISB for varying intervals of time before the final thirty-minute incubation period with casein. It can be seen that there is little decrease in the optical density produced by thrombolysin incubated zero minutes from thrombolysin incubated thirty minutes before the mixture is placed on a casein substrate. The middle broken line shows the optical density produced when thrombolysin is incubated with the preoperative plasminogen-free plasma (prepared in a manner described by Norman⁹) for varying intervals of time prior to the final

thirty minute reaction with casein. The progressive decrease in optical density as the incubation time is prolonged illustrates the inhibitory substances to thrombolysin proteolytic activity present in the preoperative plasma. The lowest line illustrates the results of incubation of thrombolysin with plasma taken from the same patient seven days after operation. Much greater inhibition to thrombolysin activity is present in the postoperative sample than in the preoperative one.

Effects of Thrombolysin Therapy on Fibrinolytic System: Table II illustrates, in a few representative patients, the modifications in plasma inhibition, as well as plasma fibrinogen concentration, euglobulin lysis times and undiluted and di-

luted plasma lysis times produced by thrombolysin therapy. There is an inverse relationship between fibrinolytic activity (demonstrated by the euglobulin and plasma lysis times) and the antithrombolysin (inhibitor) activity. The fibrinogen concentration of the plasma drops also if the dose of thrombolysin administered has been sufficiently large, but this test will not always reflect the changes in the antithrombolysin activity produced in the plasma by such therapy.

COMMENTS

From the data presented it is apparent that there are factors in the plasma of patients with thromboembolic disorders and myocardial infarction which inhibit the lysis of a fibrin clot by thrombolysin. Inhibition to the proteolytic activity of thrombolysin is also present. There was a parallel rise in the plasma fibrinogen concentration in all instances in which this increased inhibition was observed.

By studies using I181 fibrinogen as a substrate and differential titration, Shulman concluded that plasmin inhibitors were different entities from trypsin inhibitors.10-12 He also found that the plasmin inhibitors could be precipitated from the plasma at lower ammonium sulfate concentrations and were more stable to heat than the trypsin inhibitors. Norman and Hill,18 however, using starch electrophoresis combined with proteolytic assay on casein, found there were two plasmin inhibitors: (1) a slow or heat labile alpha-1 globulin, combining with plasmin non-dissociably and at a rate dependent on temperature; and (2) an immediate or heat stable alpha-2 globulin, which combines dissociably with plasmin independently of temperature. We believe that the antiplasmin described by Shulman was probably identical with their immediate or heat stable antiplasmin and that much of Shulman's trypsin inhibitor would correspond to their alpha-1 or slow inhibitor. If the latter assumption is correct, much of the thrombolysin inhibition found in our studies would correspond to Shulman's trypsin inhibitor. In both instances the rise of inhibitor activity parallels the rise in plasma fibrinogen, and factors causing a drop in plasma inhibitor also reduce the plasma fibrinogen.

SUMMARY

A semi-quantitative method was devised to determine thrombolysin inhibitor activity in human plasma. This was determined in normal control subjects, in patients with acute myocardial infarction and in patients with various thromboembolic disease states, as well as before and after the administration of thrombolysin.

Increased inhibition to the plasmin-plasminogen system was found in the plasma of patients with acute myocardial infarction and thromboembolic disease.

The plasma fibrinogen concentration was elevated in all instances in which increased inhibitors were found in the plasma.

Both plasma inhibitors and plasma fibrinogen concentrations were lowered following the administration of thrombolysin.

The inhibitors were believed to be identical with the trypsin inhibitors reported by Shulman and possibly with the slow inhibitor reported by Norman and Hill.

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DISCUSSION OF PAPER BY DRS. SANDBERG, TSITOURIS AND BELLET

DR. BERNARD J. HAVERBACK (Los Angeles, California): I have found Dr. Sandberg's statement that

patients with thrombotic or thromboembolic disease have an increase in the total serum plasmin inhibitor of considerable interest. However, our studies have revealed that the alpha-1 globulin trypsin inhibitor rises markedly in various diseases. If the alpha-1 globulin plasmin inhibitor is the same or a similar substance as the alpha-1 globulin trypsin inhibitor, this rise may not be specific for thrombotic disease states.



Comparative Effectiveness of Intravenous and Intra-arterial Fibrinolysin Therapy*

PAUL W. BOYLES, M.D., WILLIAM H. MEYER, M.D., JACK GRAFF, CATHERINE C. ASHLEY and ROBERT G. RIPIC

Miami, Florida

FIBRINOLYSIN is a naturally occurring enzyme from the blood which dissolves intravascular fibrin. Inhibitors in the blood will rapidly inactivate systematically administered fibrinolysin; however, studies in animals have indicated that these inhibitors can be exceeded temporarily by infusions of large quantities of fibrinolysin. Preliminary clinical observations on fibrinolysin therapy have been encouraging. Application of this approach to restore the coronary or cerebral circulation soon after the onset of thrombosis could reverse some instances of myocardial or cerebral ischemia and even prevent or limit the extent of damage.

Previously, we described an in vivo method for the evaluation of fibrinolysin utilizing radio-paque blood clots. The intravenous administration of human fibrinolysin accelerated the lysis of these blood clots induced in the veins of dogs. ⁵

The present investigation compares intravenously and intra-arterially administered fibrinolysin in the lysis of induced coronary artery and carotid artery thrombi in dogs. The rate is determined conveniently by serial x-ray examinations of the induced clot. Some data on different methods for determining fibrinolytic activity and histological studies are discussed.

MATERIAL AND METHODS

Adult mongrel dogs, weighing 6.8 to 18 kg., were anesthetized with intravenously administered sodium pentobarbital (120 mg./kg. body weight) and placed in the right lateral position on the operating table. The chest wall on the left side was opened in the fifth intercostal space; respiration was maintained artifi-

cially by a neophore respirator pump at a rate of 15 to 18/minute. The pericardium was exposed through the retracted ribs and a branch of the left descending coronary artery was temporarily occluded with two silk sutures placed approximately 2 mm. apart. Artery segments of approximately equal size and diameter were selected in each dog and the distal end of the occluded artery segment was injected with 0.2 ml. of an equal volume mixture of human thromboplastin† and a radiopaque media (Dionosil®).‡

The mixture was given through a 23 gauge scalp vein needle which was attached via polyethylene tubing to a small syringe. Within twenty-five minutes a blood clot formed in the occluded artery which had radiopaque material trapped in the clot (Fig. 1). The needle was removed from the artery after the formation of the clot to prevent bleeding from the puncture site in the arterial wall. Subsequently, both silk sutures were removed from the artery. In later experiments the distal sutures were left intact to prevent dissemination of the clot to the terminal branches. X-ray examination confirmed the presence of the radiopaque coronary thrombi and the chest wall was closed. In two dogs the fate of the clots was determined by direct visual observation and palpation of the clot through the open chest wall. The fate of the coronary clots was determined by serial x-ray examination. One hour after the formation of the clots the dogs were treated with 200 to 500 ml. of intravenously or intra-arterially administered fibrinolysin dissolved in saline.§ The control ani-

† Prepared as described by Quick¹⁰ (heated at 80 °c. for ten minutes).

‡ Contains 60 per cent W/V prophyliodone (N-propyl ester of 3:5 diiodo-r-pyridone-N-acetic acid) suspended in aqueous solution. Glaxo Laboratories, Ltd., Greenford, England.

§ Human fibrinolysin A, Ortho Pharmaceutical Company, Raritan, New Jersey. Human fibrinolysin B, Merck Sharp & Dohme, West Point, Pennsylvania.

^{*} From the Medical Research Division, V. A. Hospital, Coral Gables; the Departments of Medicine and Surgery, University of Miami School of Medicine, Miami; and the Coagulation Research Laboratory, Miami Heart Institute, Miami Beach, Florida. This study was aided in part by grants from the Florida Heart Association and the U. S. Public Health Service, National Institutes of Health, Grant No. H-3951 (R1).

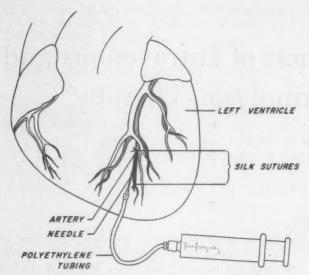


Fig. 1. Schematic illustration of the method for the production of radiopaque coronary thrombi.

mals were given saline intravenously. The fibrinolysin units were determined by the manufacturer. Serial electrocardiographic recordings and serum glutamic oxalacetic transaminase (SGOT) levels were obtained in most of the dogs to estimate the extent of myocardial damage.

Radiopaque clots were induced in the internal carotid arteries in dogs² by placing clamps on a 2-inch segment and injecting the occluded artery with 0.5 ml. of the thromboplastin radiopaque material. After one hour the clamps were removed. The clot became a radiopaque embolus or emboli to the head. Serial x-ray studies permitted assessment of the effects of therapy with human fibrinolysin given intravenously or directly into the involved artery.

Prothrombin time, thromboplastin generation and two-stage prothrombin assay were performed by described methods. Fibrinogen was determined by the biuret method on isolated plasma clots. Esterase activity was estimated by the TAME assay method of Sherry with p-toluenesulfonyl-1-arginine methyl ester as the substrate. The clot lysis time on a standard clot with varying dilutions of serum, fibrin plate with purified 0.33 per cent bovine fibrinogen and gel agar diffusion plates with specific human fibrinolysin antiserum were used to determine fibrinolytic activity. SGOT activity was determined by the method of Karmen et al. 15

RESULTS

Fate of Coronary Thrombi as Determined by X-Ray Films: The x-ray films of a dog treated with intravenously administered human fibrinolysin are shown in Figure 2. The x-ray appearance of the clot at the time of surgery, after closure of the chest wall, four hours and ten days after the infusion of fibrinolysin, is shown in Figure 2. Complete lysis of the radiopaque clot has

occurred in this period of time. Partial lysis of the clot resulted in dissemination of the clot into the terminal branches of the artery (Fig. 3). In the control animals the radiopaque clot can be demonstrated by x-ray examination for six to ten days. Several dogs were treated with fibrinolysin administered into the root of the aorta under pressure. It was found that most of the clot was dissolved within four to six hours (Fig. 4). Different stages in the formation and organization of the coronary thrombi in three dogs at different times are shown in Figure 5.* It will be noted that these clots are quite "physiological" and have relatively little of the radiopaque material entrapped within. In many of the dogs ventricular fibrillation has developed with lysis of the clot; this can be partially prevented with procaine amide therapy.

Fate of Cerebral Emboli as Determined by X-Ray Films: Figure 6 illustrates the failure of intravenously administered fibrinolysin to dissolve a radiopaque carotid artery clot. At necropsy a large area of infarction without gross hemorrhage was noted (Fig. 7). Complete lysis of another carotid artery clot is shown in Figure 8 within four hours after administration of human fibrinolysin directly into the involved artery.

Blood Studies: Hemorrhage has been a major problem in the animals treated with 500,000 units or more of fibrinolysin, whether given intravenously or intra-arterially. Serial blood studies reveal that the fibrinogen and prothrombin fall to non-assayable levels within one-half and four hours, respectively, after the administration of 500,000 units of either fibrinolysin A or B. Slightly enhanced levels of circulating fibrinolytic activity can be demonstrated by several assay methods (clot lysis with diluted serum, TAME assay and fibrin plate lysis) at different times after the administration of fibrinolysin, which suggests that these tests are measuring different functions or actions. Recently, the use of dilutions of serum with a gel agar diffusion technic (Fig. 9) has enabled us to demonstrate immunologically an increased titer of circulating fibrinolysin in dogs after treatment with human fibrinolysin. This increased titer lasted for about three hours. This technic appears to be useful as a guide in the clinical

^{*} The histological sections were obtained through the courtesy of Dr. Jerome Benson, Mount Sinai Hospital, Miami Beach, Florida.

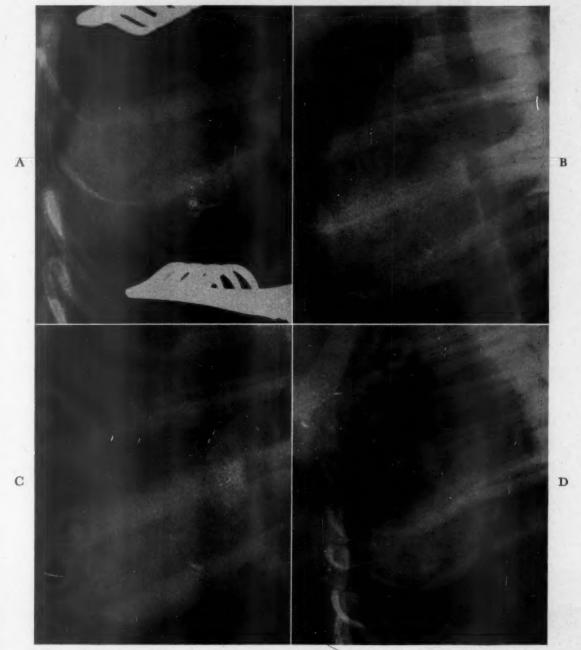


Fig. 2. Serial x-ray films of a dog treated with intravenously administered fibrinolysin. A, at the time of surgery. B, after closure of the chest wall and before the administration of fibrinolysin. C, four hours after the intravenous administration of fibrinolysin. D, eleven days after the intravenous administration of fibrinolysin.

administration of fibrinolysin in preliminary observations.

COMMENTS

In the data presented, fibrinolysin administered directly into the artery significantly accelerates the lysis of induced radiopaque arterial thrombi. Intravenously administered fibrinolysin was not effective in the studies reported. These findings are in marked contrast to previous results with intravenously administered fibrinolysin in the lysis of radiopaque venous clots.¹¹

The indefinite and variable results obtained with several different methods for the assay of circulating levels of fibrinolytic activity agree with recent reports 16.19 and emphasize the need for a simple and accurate assay method as a



Fig. 3. Picture of the heart from a dog treated with intravenously administered fibrinolysin. Note the radiopaque clot fragments in the terminal branches of the artery. From: Boyles, P. W. Antithrombotic Therapy. New York, 1959. Grune & Stratton.¹¹



Fig. 4. Serial x-ray films of a dog treated with segmental fibrinolysin administered into the root of the aorta under pressure. A, at the time of surgery. B, before treatment. C, one hour after starting treatment. D, three hours after starting therapy. E, four hours after starting the infusion. Note almost complete lysis of clot within this period of time.

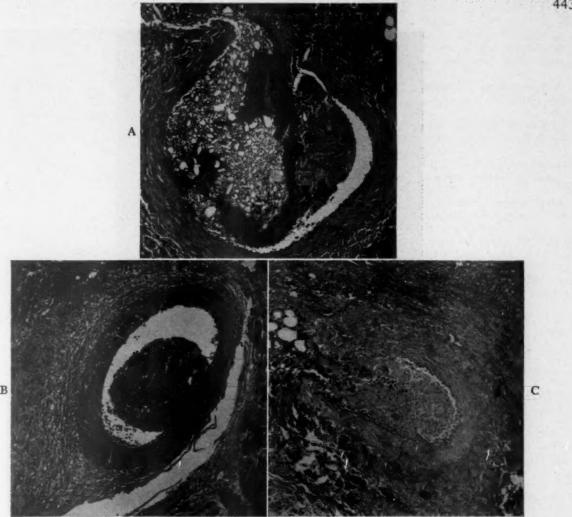


Fig. 5. Sections of thrombus within branches of left descending coronary artery (original magnification \times 66). A, portion of recent thrombus in major coronary artery with radiopaque material in the clot, age twenty hours. B, early thrombus in small artery of myocardium, age twenty hours. C, organized thrombus with recanalization appearing as polypoid mass within lumen, age seventeen days.

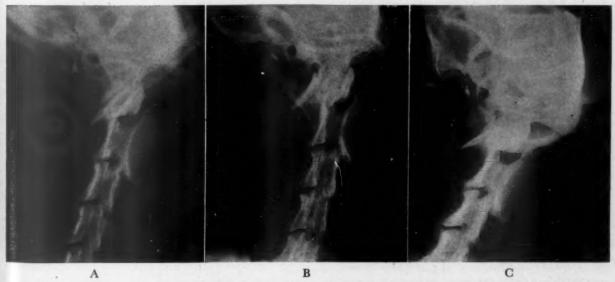


Fig. 6. X-ray films showing radiopaque clot in carotid artery unaffected by intravenously administered fibrinolysin. A, before treatment. B, three hours after treatment. C, fourteen days after intravenous therapy.



Fig. 7. Picture of the gross appearance of the brain of dog (Fig. 6) treated with intravenously administered fibrinolysin.

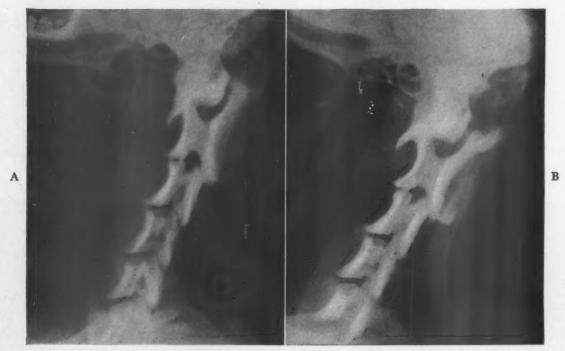


Fig. 8. Serial x-ray films of radiopaque clot in carotid artery. A, before treatment. B, after three hours of infusion of fibrinolysin directly into the artery under pressure, showing almost complete lysis of the clot.

guide for the use of fibrinolytic therapy. ¹⁸ The marked destruction of fibrinogen and prothrombin with massive fibrinolysin therapy ¹⁷ indicates the proteolytic effects of the preparations and suggests that these dosages are beyond the limits that may be safely infused into patients. The

finding that intra-arterially administered fibrinolysin is more effective in the lysis of arterial clots in the experimental animal is probably related to the speed and amount of active enzyme or activator reaching the clots before inactivation with the inhibitors in the blood and tissues.



Fig. 9. Picture of gel agar diffusion plate: wells 1 and 8, fibrinolysin B; wells 6 and 9, fibrinolysin A; wells 3 and 5, streptokinase; well 2 contains rabbit antiserum to fibrinolysin B; well 4 contains rabbit antiserum to streptokinase; and well 7 contains antiserum to fibrinolysin A. This demonstrates the relative purity of fibrinolysin A and the mixture of materials in fibrinolysin B.

Indeed, it may be that no demonstrable enhanced level of circulating fibrinolytic activity is necessary to have acceleration of *in vivo* lysis of intravascular fibrin due to the adsorption of fibrinolysin and/or activator onto the fibrin clot which can occur with segmental administration of fibrinolysin.

SUMMARY

The rate of lysis of induced coronary and carotid artery thrombi is not significantly affected by the intravenous administration of human fibrinolysin. Intra-arterial administration was successful. These findings indicate that segmental injection of the fibrinolysin near the site of the clot is necessary to obtain effective in vivo lysis of arterial thrombi.

Preliminary observations support the view that the intra-arterial route is the mode of choice for administering fibrinolysin in the treatment of acute arterial thrombosis or embolism.

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DISCUSSION OF PAPER BY DRS. BOYLES, MEYER, GRAFF, ASHLEY AND RIPIC

DR. CARLOS GROSSI (New York, New York): At the Sloan-Kettering Institute in 1953 and 1954, we produced thrombi in femoral arteries of rabbits, cats and dogs, by sodium morrhuate sclerosing technic, and studied the effect of human fibrinolysin on these thrombi at twenty-four and forty-eight hours of age. Direct arterial visualization and biopsy or autopsy were used. It was our experience that intravenous administration of human fibrinolysin in the dosage

of 6,000 to 9,000 units/kg. of body weight over a period of approximately four to eight hours resulted consistently in the dissolution of intra-arterial thrombi in over 90 per cent of our animals. Why do our results differ? I believe that the method of production of the thrombi is different and that perhaps the use of sodium morrhuate in local trauma simulates in some fashion the atherosclerotic changes with local thrombosis in man. We did find that when we gave intra-arterial fibrinolysin through a catheter proximal to the experimentally produced thrombus the lysis time of this thrombus in the femoral artery of dogs was in the range of two hours instead of six to seven hours. This may be of possible clinical importance in its use in man. We have demonstrated consistently, by the administration of 4,000 to 5,000 units/kg. of human fibrinolysin in various animals, euglobulin activity of the order of twenty minutes to forty-five minutes; we have also found fibrinolytic activity of the whole clot lysis of the order of one to two hours. As for the local oozing from an open wound during the administration of fibrinolysin, we have found this to be true when the animal has a fresh wound.

In conclusion, I would like to mention that we have been interested in human fibrinolysin in the treatment of arterial thrombi in man and have used it both intravenously and intra-arterially. In one instance, a patient with a thrombosis of the ulnar artery was given approximately 8,000 units/kg. of body weight of fibrinolysin intravenously; in four hours there was lysis of the ulnar artery thrombus. We have had other instances in which we have combined human fibrinolysin therapy intra-arterially at the time of embolectomy. In this connection, it must not be forgotten that an embolus is not a thrombus. In one case in which we removed an embolus from a femoral artery, we placed that embolus in fibrinolysin solution and incubated it. We were not able to lyse it in vitro over a period of forty-eight hours; on the other hand, we have had experiences in which a distal propagated clot in the arterial tree is lysed by the direct administration of fibrinolysin, and when 200,-000 units of fibrinolysin are given intra-arterially over thirty minutes to subjects with open operative wounds, there is no oozing from the wound.

DR. ALVIN FREIMAN (New York, New York): About three years ago I started to work with radio-paque substances and encountered several problems. We were unable to find a satisfactory radiopaque medium for two reasons. Using radiopaque particulate matter we found that it lodged in one portion of the clot. A portion of the clot was formed which therefore could not be demonstrated on x-ray. Secondly, by the use of some of the radiopaque solution we found that in vitro there was a marked increase in antiplasmin activity, i.e., a marked inhibition of lysis when exposed to plasmin. When we formed radiopaque clots in animals we found a marked inflammatory reaction in the vessel wall much more than we had found with any non-opaque clot. Finally,

with such a clot, we were able to visualize only that portion of the clot that we had marked originally. The subsequent clot that was formed or reformed was not demonstrated by that type of procedure.

DR. PAUL W. BOYLES (Miami Beach, Florida): Dr. Grossi has commented on the induction of a blood clot with sodium morrhuate which produces local tissue damage. It is true that this type of clot is different from the one we produced and, perhaps, some of the difference in the observed rate of lysis can be related to the tissue damage which may activate locally the fibrinolytic system. Tissue extracts have been shown to be very potent activators of fibrinolysin.

The production of experimental clots with serum has theoretical objections because serum or serum fractions may be contaminated with large quantities of proactivators and/or activator which may accelerate the rate of clot lysis. Thromboplastin can be heated to eliminate these contaminants while retaining a good deal of its thromboplastic activity. These factors may be important differences in the observations that we have obtained compared with

those reported in the literature.

Clinically, we have had little success in achieving any significant improvement with either intravenous or intra-arterial fibrinolysin in the treatment of arterial emboli composed of "old clot material," particularly in patients with emboli who have atrial fibrillation associated with rheumatic heart disease. In patients with "fresh" arterial thrombosis or emboli rapid lysis of the clot has been observed with direct intra-arterial administration of the fibrinolysin. I am glad to hear that this has been the experience of Dr. Grossi and the group at the Sloan-Kettering Institute.

Dr. Freiman's findings on the effects of different radiopaque materials in experimental blood clots are similar to those we encountered in selecting a suitable radiopaque material for experimental purposes. Most of the commercial preparations are rapidly absorbed from the clot and produced local inflammatory effects similar to that seen with sodium morrhuate. We finally utilized a radiopaque material which was available in an aqueous suspension and caused no significant local inflammatory response. This preparation is called Dononsil® (Glaxo Laboratories, Ltd., Greenford, England).

Radiopaque thrombi are useful in studying in vivo clot lysis as partial lysis of the clot may occur and would be lost in the circulation except for the fact that it is radiopaque. In vitro studies demonstrated no significant alteration in the spontaneous whole blood lysis time with this radiopaque material trapped in the clot. This, of course, does not mean that the fibrinolysin activity may not have been inhibited, because we believe that the spontaneous lysis time is a crude method of demonstrating fibrinolytic activity. At present, we are not certain which is the best method for demonstrating fibrinolytic activity; however the fibrin plate method is very reproducible.

Panel Discussion: Assay Technics

The Problems of Correlation with Results of Treatment

Moderator: WERNER BAUMGARTEN, PH.D., West Point, Pennsylvania CLARA M. AMBRUS, M.D., PH.D., Buffalo, New York KEITH B. McCALL, PH.D., Lansing, Michigan ROBERT B. PENNELL, PH.D., Cambridge, Massachusetts

STANDARD CLOT LYSIS TECHNIC

DR. BAUMGARTEN: At present, we have methods to determine the potency of fibrinolysin preparations. However, a suitable reference preparation for standardization of fibrinolysin is required. Such a standard preparation is not available. In dealing with fibrinolytic agents derived from different sources and prepared by different technics, a universal standard probably will not be possible.

Figure 1 demonstrates that fibrinolytic agents prepared in different manners will not necessarily give the same dose response as judged by clot lysis activity in vitro. At the top of this graph is plotted the standard, a streptokinase-activated human plasminogen. The type 1 preparation is also a streptokinase-activated human plasminogen, plotted separately to permit comparison with the standard. The type 2 preparation is a chloroform-activated bovine plasminogen which gives a different dose response curve. It is apparent that, in a fibrinolytic assay, a different reference standard must be used for each type of preparation.

A fibrinolytic assay, that is, an assay depending on the dissolution of a clot, is more suitable for evaluation and standardization of fibrinolysin preparations than procedures which depend on substrates other than fibrin. We have had experience with a number of test systems in which we measured hydrolysis of casein and of various synthetic esters and found all these tests less desirable than a fibrinolytic assay because of the unique interrelationship of "activator" and "plasmin" activities in human fibrinolysin preparations.

It is important to accurately measure fibrinolytic activity in the laboratory in order to assure that fibrinolysin preparations will give a predictable response in man. This is best achieved by clot lysis assay technics.

Description of Various Assay Methods

DR. Ambrus: For each type of plasmin preparation, a specific test has to be worked out and each preparation must be tested for several activities. For example, therapeutic results can be obtained with preparations of high activator and relatively low plasmin activity, or with preparations of high plasmin and relatively low activator activity. If assay is carried out for only one of these parameters, one of the preparations would be unjustifiably classified as being of low potency. We have tried many methods of testing and some of our conclusions are summarized briefly:

We have found a straight fibrinolytic assay to be the simplest and most closely correlated with clinical results. Two-tenths milliliter of the assay material is mixed with 0.1 ml. purified bovine thrombin (containing 1 NIH unit) and 0.3 ml. of a 0.6 per cent solution of purified human fibrinogen in imidazole buffer at pH 7.2 at 45°c. The end point is taken as the time when all bubbles trapped in the clot rapidly

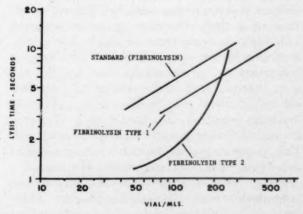


Fig. 1. Differentiation of fibrinolysin preparations by clot lysis assay.

rise to the surface. One RPMI* unit of plasmin is defined as the activity which lyses this clot in two minutes. Lysis times are converted to units on a standard curve.

Purification of Reagents: The most important factor in the assay is the purity of the reagents. Both fibringen and thrombin are contaminated with plasminogen. Thus, depending on the degree of contamination, which will vary from one batch to another, activator activity is measured to a greater or lesser extent. In order to assay plasmin activity, methods were worked out to prepare reagents free from plasminogen contamination. Laki1 described a method for the purification of fibrinogen. Repeating this procedure three times, a satisfactory reagent can be prepared from most, although not all, batches of fibrinogen. For the purification of thrombin, we used an alcohol precipitation method which was satisfactory but gave extremely poor yields. Recently, we worked out a better method with Markus,2 based on selective inactivation of the plasminogen in thrombin by \(\beta\)-mercaptoethylamine (MEA). Apparently, plasminogen activity depends on intact disulfide bonds; however, these bonds are not as essential for thrombin activity as shown by the fact that unitage of thrombin does not decrease after treatment with MEA.

Tests for Contamination: When reagents are tested for freedom from plasminogen contamination it is important to keep in mind the bellshaped curve phenomenon. When increasing amounts of streptokinase (SK) are added to a standard amount of plasminogen, the resulting plasmin activity increases, reaches an optimum and then decreases. Several preparations of varying purity of SK were tested (Varidase,® Lederle; purified SK, Lederle; purified SK, Merck Sharp & Dohme Research Laboratories); all gave essentially the same bell-shaped curve. Thus, it is likely that the inhibition obtained with high concentrations of SK is due to SK itself and not to contaminants. It is logical that when a reagent contaminated by plasminogen is tested with high doses of SK no lysis will result and it may be mistakenly concluded that the reagents are pure. It is therefore important that a dilution series of SK be used. This, incidentally, will furnish a tool to estimate the degree of plasminogen contamination.

Purity Test: The standard clot used in our laboratory is tested for plasminogen contamina-

* Roswell Park Memorial Institute.

tion by incorporating into clots concentrations of SK varying from 0.1 unit to 1,000 units. Clots prepared with crude reagents will lyse in as little as twelve minutes with 100 units of SK. In contrast, the standard clot made with purified reagents will show no lysis or will not lyse for ten or more hours. These are minimal lysis times obtained with "optimal" SK concentrations as mentioned above. The optimal concentration is usually 10 units of SK per clot in the purified standard clot. With lower or higher SK concentrations, the clots remain stable for several days. The estimated amount of plasminogen in the purified clot is so small that it cannot be expressed in units on our standard curve. It is considerably less than 0.01 RPMI unit per clot.

This purity test is applicable only to human fibrin clots, since bovine plasminogen is not activated by SK except in the presence of human proactivator.

Assay of Plasmin and Activator Activity: When assaying a plasmin preparation, it is important to employ a dilution series and base conclusions on the dilutions resulting in lysis times most closely approximating two minutes.

Activator activity can be tested by mixing a dilution series of a plasmin preparation with a standard amount of plasminogen and then determining the resulting plasmin activity. The difference between plasmin activity in the mixtures and the activity of control preparations of plasmin alone will give the units of activator activity.

Clinical Correlations: Correlating our assays with clinical results in acute thrombophlebitis, we concluded that 15 to 30 RPMI units of plasmin activity per kg. body weight will result in clinical improvement in about 80 per cent of the patients.^{8,4} The same dose levels will produce lysis in the I¹⁸¹-labeled clot technic as performed by Back in our laboratory^{8,6} in dogs *in vivo* in about 95 per cent of the animals.

Correlating activator activity with therapeutic results is more difficult since this will depend on the plasminogen level of the patient, the composition and structure of the clot, duration of the infusion and other factors.

MEASUREMENT OF FIBRIN DISSOLUTION

DR. PENNELL: We share Dr. Ambrus' view-point and that of Dr. Baumgarten that the fibrinolytic assay would appear to give the most valuable information as to possible clinical effect of a

particular preparation. The procedure has just been adequately discussed and some of the problems emphasized. There is a great need for a standard unit.

Types of Assay: Plasmin possesses a variety of lytic properties, measurement of which can be used for assay of plasmin per se or of plasminogen after its conversion to plasmin. It dissolves fibrin, makes fibrinogen incoagulable, lyses benzoyl- and tosyl-arginine methyl esters and lysine ethyl ester and hydrolyzes casein. Not only are many of these properties shared by other enzymes derivable from plasma proteins, but it also has been reported that the esterase and the caseinolytic activity of plasmin preparations need not correlate with their fibrinolytic or fibrinogenolytic activity. Since fibrinolysis is the only clinical result desired, the assay should be based on this phenomenon.

Comments on the Measurement of Fibrin Dissolution: A suitable assay must be sufficiently reproducible to permit valid comparison, not only of measurements taken over a period of time in a single laboratory, but also of measurements made in many different laboratories. The assays based on esterase and caseinolytic activity are attractive in that standard, simple, objective measurement of the process of lysis can be made. These assays seem inadequate for other reasons, however, as already mentioned. Measurements of fibrinolysis, on the other hand, have usually depended on a subjective measurement, namely, the visual observation of the disappearance of the clotted fibrin. Obviously, assessment of such an end point will vary not only from one laboratory to another but also with time in any one laboratory and even with any one assayer. An attempt to accommodate this problem is being made in our laboratory by the use of an instrument which provides a continuous record of the turbidity of a solution. Typical tracings are illustrated in Figure 2. Clot formation and lysis are both recorded by the instrument. Unitage may be calculated from lysis times read as the interval between the beginning of opacity decrease and the point of return to the baseline, or from lysis times read with more sensitivity when reduction in opacity is 50 per cent of the maximum value.

Bovine fibrinogen, being readily available commercially, has most generally been used in the fibrinolytic assay. Its use has proved satisfactory, but two aspects are worthy of comment. A problem could arise from the

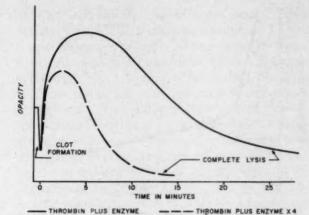


Fig. 2. Clot formation by thrombin and lysis by fibrinolysin.

variability of the clottable protein in different lots of commercial bovine fibrinogen. In a routine examination of ten lots, clottable protein has been reasonably constant between 72 and 82 per cent of the total protein for eight of the ten samples. The other two showed 62 per cent and 57 per cent clottable protein, respectively.

Bovine plasma lacks the activator essential for the conversion of plasminogen to plasmin by means of steptokinase. The use of bovine fibrinogen, therefore, cannot be expected to give any indication of the presence of unreacted streptokinase in a plasmin preparation. Such unreacted steptokinase would probably contribute to the over-all *in vivo* fibrinolytic effect of the product.

Human fibrinogen is also available commercially but not in the form most adaptable for use as a laboratory reagent. Examination of an appreciable number of human fibrinogen preparations in our laboratory has shown relatively little variability in clottable protein content between lots from a single supplier. However, there may be appreciable difference in this respect between suppliers.

Theoretically, the use of human fibrinogen should have the advantage, from the standpoint of assay of total fibrinolytic potential, of detection of the presence of proactivator. After this, the presence of unreacted streptokinase in a plasmin preparation could be assessed. Direct comparison in our laboratory of assays using bovine fibrinogen with assays using human fibrinogen, both performed with the same plasmin preparation, have shown differences in lysis time, the lysis time with human fibrinogen being somewhat faster. The differences were

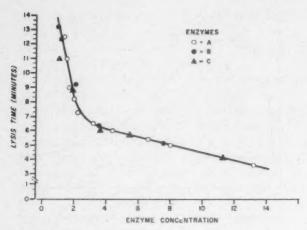


Fig. 3. Dose response curve of several fibrinolysin preparations.

slight, however, and suggested either that little unreacted streptokinase was present in the preparation, or that the increment of lysis due to streptokinase is relatively insignificant.

Perhaps the most important reagent needed for reliable assays is a standard plasmin preparation of assigned unitage. Such a standard, recognized as such by the various laboratories concerned, does not exist at present. Use of such a standard or of any plasmin preparation whose potency relative to the standard is known, as a part of each series of assays, would minimize the importance of variability in fibrinogen lots. It would appear that definition of the unit to be used for such a standard is of secondary importance. Laboratories wishing to use a unit differing from that of the standard could still refer their preferred unit to the standard. Equally as important as the standardization of the assay is the assurance that the clinician is not presented with preparations of different origin, the declared unitage of which may bear little or no relation to one another.

Examination of the shapes of curves obtained by plotting enzyme concentration against lysis time reveals remarkable identity of results. In Figure 3 the data for three plasmin preparations have been superimposed on one another. A single curve can be seen to fit equally well with the three sets of observations. Enzymes A and B are individual preparations from the same source. Enzyme C is a preparation from a different source.

Although the aforementioned similarity of different plasmin preparations is the usual experience, it is possible to prepare the enzyme in such a way that this relation is lost. One variant encountered results in appreciable

spread of values at moderate concentrations of the enzyme, but as the lysis time decreases with increased enzyme concentration, the deviant curve approaches that of the usual curve. It is probable that it is in the region of moderate dilution that the most accurate comparison of fibrinolytic potential is obtained. It is undoubtedly important that assays within a specified unitage range be included in any assessment of fibrinolysis.

IDENTIFICATION, QUANTITATION AND CLASSIFICATION OF FIBRINOLYTIC AGENTS

Dr. McCall: The following material on assay methods as developed in our laboratory and problems attendant on them will be submitted for publication by Drs. J. T. Sgouris, J. K. Inman, H. D. Anderson and myself.*

The approach to management of thromboembolic disorders by the dissolution of clots in blood vessels has gained impetus in the last decade. A number of fibrinolysin preparations have been, or are about to be, introduced for clinical evaluation. These preparations, although showing fibrinolytic activity in vivo, differ considerably in regard to their origin and mode of action; consequently, in order to ascertain what a given preparation may be expected to accomplish when used clinically, the nature of the active materials and their concentrations must be known. It is my purpose to present a systematic approach to the identification and quantitation of these agents, and to propose a classification of these materials based upon their source and type of action.

FIBRINOLYTIC ASSAYS (QUALITATIVE)

Test A: This test is designed to detect direct fibrinolytic activity on heated human fibrin plates. Fibrin thus treated has been shown to be free of intrinsic profibrinolysin. The plates are prepared by the Sgouris, Inman and McCall⁸ modification of Lassen's⁹ technic. The tests are conducted by placing single drops of the samples on a plate and incubating the plate at 32°c. for eighteen hours. The reaction is stopped by flooding the plate with 10 per cent (w/v) trichloroacetic acid. A lysed (cleared) area (+) indicates the presence of direct fibrinolytic activity. Streptokinase (SK), strepto-

^{*} This work was carried out at the request of the Director of the American National Red Cross Blood Program under an agreement between the American National Red Cross and the Michigan Department of Health Laboratories.

kinase-globulin complex, urokinase and profibrinolysin do not, by themselves, show this

direct lytic activity.

Test B: This is designed to show the presence of profibrinolysin activators. The plates required are prepared as in test A8,9 with the omission of the heating period at 80°c. Activators of human profibrinolysin such as streptokinase, urokinase or the Müllertz¹⁰ streptokinase-globulin combination, show lysis of fibrin in this test (+) by activating the profibrinolysin associated with the human fibrinogen used. If some lytic activity is present in test A and if activator is also present, a larger lysed area (++) is observed in test B than in test A when identical samples are tested.

Test C: This test is designed to show the presence of profibrinolysin. One-tenth milliliter of sample solution is added to 0.2 ml. of streptokinase (3,000 units per ml.) at 37°c. and single drops of the solution are placed on the heated fibrin plates as in test A. A lysed area in this test indicates the presence of profibrinolysin in the sample if tests A and B are negative. Also, an increased area of lysis over that in test A, test B or both, also indicates the presence of profibrinolysin in the sample.

Test D: This test is designed to differentiate urokinase or streptokinase-human globulin complex from streptokinase alone through the ability of the sample to activate bovine profibrinolysin. Streptokinase alone does not activate the bovine profibrinolysin. This test is identical to test B except that bovine fibrinogen is substituted for human fibrinogen in the preparation of the unheated fibrin plates. Typical results of the use of the qualitative tests (Table 1) are discussed later.

Confirmation and extension of the results of the qualitative tests (A through D) and determination of relative potencies are possible through the use of the following caseinolytic assays.

CASEINOLYTIC ASSAYS (QUANTITATIVE)

Test E: In this test, direct caseinolytic activity is quantitated by our modification¹¹ of the caseinolytic method of Remmert and Cohen. 12 In this assay 0.1 ml. of the test sample is placed in a test tube and diluted to 2 ml. with the designated buffer (pH 7.4; 0.1 M phosphate-saline). Six per cent casein solution (4 ml.) is added, mixed, and the mixture is placed in a 37°c. water bath. A 2 ml. aliquot is then taken out immediately (zero time) and

another is removed sixty minutes later. Each aliquot is added to an equal volume of 10 per cent trichloroacetic acid. Then 3 ml. of water is added, and the mixture is filtered through Whatman No. 2 paper. The optical densities of the filtrates are read against a water blank at 280 mu. The difference in optical density from zero to sixty minutes is converted to micrograms of acid-soluble tyrosine by reference to a standard curve. One caseinolytic unit (C.U.) has been arbitrarily taken as the amount of fibrinolysin producing an increase of 450 µg. of acid-soluble tyrosine in a 4 per cent casein medium in one hour at 37°c. Each sample is run in triplicate and the assay quantitated in terms of caseinolytic units per ml. (C.U. per

ml.) or C.U. per vial.

Test F: This test is an assay of activator concentration, using highly purified human profibrinolysin. A mixture is prepared containing an excess of substrate (human profibrinolysin; 23 C.U. per ml.) in 0.5 ml. volume, together with 0.1 ml. test sample, 1.4 ml. phosphatesaline buffer and 0.3 ml. 6 per cent casein solution. This mixture is placed in a 37°c. water bath for a ten-minute activation period after which 3.7 ml. of casein solution is added. A 2 ml. aliquot is then withdrawn immediately (zero time) and mixed with an equal volume of trichloroacetic acid. A second 2 ml. aliquot is withdrawn after sixty minutes at 37°c. The test is concluded and units calculated in the same manner as for test E. In this case, any casein digestion is expressed as caseinolytic (activator) units per ml. or per vial (if test E is negative). However, if test E is positive then the difference between test F and test E represents the activator content of the preparation. This test does not differentiate among the several activators; thus the activity measured is due to any one or more of the following: streptokinase, urokinase, tissue activators, streptokinase-globulin complex, etc. If the preparation is solely fibrinolysin, then test E and test F give identical results. By extending the incubation time a stoichiometric and an enzymatic activation process may be distinguished from one another, thus further delineating the type of activator present.

Test G: This test is designed to quantitate the difference between urokinase or streptokinase-human globulin complex and streptokinase alone through the ability of the sample to activate bovine profibrinolysin as measured in the caseinolytic assay. Test G is identical to test

Table 1

Typical Results of the Tests A to I as Applied to Different Types of Agents Proposed for in Vivo Thrombolysis

	Qualitative Tests				Quantitative Tests*				
Agent	A	В	C	D	Е	F	G	Н	I
Human fibrinolysin Human fibrinolysin	+	+	+	+	21.2	22.7	18.1	21.8	21.
plus "activator"†	+	++	+	++	21.2	33.7	31.6	33.1	33.
Bovine fibrinolysin	+	+	+	+	5.2	5.3	4.7	5.8	5.
"Activator" alone	-	+	_	+	0.2	13.3	14.2	0.2	13.
Human profibrinolysin	-	-	+	_	-	-	_	12.8	12.
Streptokinase		+	_	_	-	15.0	-	-	-
Urokinase	-	+	-	+	-	13.4	11.6	-	8.

* Caseinolytic units per milliliter.

† In this case "activator" refers to a streptokinase-globulin complex. Due to the limiting quantities of "activator," a smaller amount was used in tests H and I and therefore these tests cannot be directly compared to tests E, F and G.

F except that bovine profibrinolysin is used. Any measurable activity shown by this test indicates the presence of urokinase or streptokinase-human globulin complex.

Test H: The presence of human profibrinolysin can be determined by the following test. One-tenth milliliter of sample solution and 0.2 ml. of streptokinase (containing from 100 to 3,000 units per ml.) are diluted to 2 ml. with phosphate-saline buffer; then 0.3 ml. casein solution is added. This mixture is incubated for ten minutes at 37°c, to provide for the conversion of any profibrinolysin to fibrinolysin. Following this, 3.7 ml. of casein solution are added. Two milliliter aliquots are withdrawn immediately (zero time) and after sixty minutes, and all are treated in the same manner as in test E. Activity or increased activity over that measured in test E indicates and measures the presence of profibrinolysin in the preparation.

Test I: Inasmuch as the presence of a "human globulin" is required for the action of SK on bovine profibrinolysin, a test for that globulin may be useful. A test for human globulin is conducted by adding streptokinase (0.2 ml.) to the test samples and otherwise following test G. If test G is negative and test I positive, then the presence of human globulin and the absence of streptokinase are indicated.

Representative results with various types of preparations are shown in Table 1.

Test J: If it is desirable to differentiate urokinase from the SK-globulin complex, the following method may be employed: The sample is adjusted to pH 8 with sodium hydroxide and held for ten minutes to destroy any uropepsin which may be present as an impurity in the urokinase. Then the solution is lowered to pH 2 for thirty minutes at room temperature, to destroy the SK-globulin complex. Thus, any activity remaining as measured by the caseinolytic assay (test G) at pH 7.4 is due to urokinase.

COMMENTS

The fibrin plate technic used in tests A through D, for qualitative assay, is important for the identification of profibrinolysin, fibrinolysin and activators. Fibrinolysin alone is evident by its true lytic activity on the heated fibrin plates. Any preparation containing activator will show a higher level of fibrinolysis on an unheated fibrin plate than on a heated plate, since activator will convert any profibrinolysin contained in the fibrinogen to fibrinolysin and result in a greater degree of lysis. Profibrinolysin can be detected in the plate assays by the addition of SK followed by testing on the heated fibrin plate. Streptokinase alone does not result in any lysis of the heated plate, while profibrinolysis which has been activated by SK will produce lysis.

The caseinolytic assays, used in tests E through J, are important since they confirm and extend the results of the qualitative tests. They also provide a means for quantitating each of the components described. On the basis of this test system, it is possible to classify the preparations proposed for *in vivo* thrombolysis as follows:

Type I—Fibrinolysin: This consists of prepara-

tions, primarily of blood plasma origin, which have a direct proteolytic action on fibrin and a fairly high specificity toward fibrin substrates. These preparations show distinct activity on heated fibrin plates and with a casein substrate (Table 1). Example: spontaneously activated human fibrinolysin.

Type II-Agents Which Are Primarily Activators of Profibrinolysin: This consists of preparations which do not act directly on fibrin but which are intended to activate a profibrinolysin of the same origin as the fibrin substrate. The resulting fibrinolysin in turn acts on the fibrin. These "activating" preparations are subtyped according to origin as follows: (A) Type II agents prepared solely from human (or mammalian) sources. Examples: urokinase, tissue activators. 18 (B) Type II agents prepared solely from non-human (or non-mammalian) sources. Examples: streptokinase, pyrogens, certain chemical substances. (C) Type II agents prepared from certain combinations of human and nonhuman (mammalian and non-mammalian) sources. Examples: streptokinase (SK) plus a minimal amount of human globulin to form an SK-globulin complex ("activator" of Müllertz). This may occur inadvertently by addition of SK to profibrinolysin.

Type III—Combinations of Fibrinolysin and Activators: Various combinations of any of the aforementioned agents are possible. In such combinations the action of one type of agent will often predominate sufficiently to permit proper identification and classification of that agent. Examples: urokinase or SK-activated fibrinolysin etc.

Type IV—Miscellaneous: Preparations which do not fall into the aforementioned types. Examples: profibrinolysin (plasminogen), "proactivator," mold extracts, etc.

Thus, we have proposed that the term "fibrinolysin" can be reserved solely for type I preparations and that all type II preparations be designated "activators" and labelled according to origin. Type III agents should be designated as both fibrinolysin and activator, stating the number of units of each.

Summary: A method is presented for the identification and quantitative measurement of agents which have been proposed for in vivo thrombolysis. These agents are purified fibrinolysin, several types of activators, profibrinolysin or combinations of these agents. On the basis of these tests, a system of classification is proposed.

Tanza zz

In Vivo Fibrinolytic Effect of Plasmin-Antiplasmin Complex in Dogs Carrying I¹²¹-Labeled Purified Human Fibrin Clots

No. No. of of Dogs Clots	of	Treatment Intravenously	Mean % Decrease in Radioactivity of Clot			
		4 hr.	24 hr.			
2	6	Saline	6.6	12.6		
4	9	Antiplasmin alone	6.3	11.5		
4 2 4	- 6	120 units SK/kg.	2.1	28		
4	12	15 RPMI units/kg. plasmin	22.2	63		
6	18	15 RPMI units/kg. plasmin plus anti- plasmin	29	60		

Assays of Fibrinolysin System as a Guide to Treatment

DR. BAUMGARTEN: There is a question we should explore further from the clinician's point of view. Dr. Ambrus, is there a need for assays of members of the fibrinolysis system in patients as a guide to therapy?

DR. AMBRUS: Levels of plasminogen and antiplasmin (AP) are seldom low enough to contraindicate fibrinolytic therapy. We have rarely seen patients with severe hepatic failure in whom AP levels were so low that fibrinolytic therapy with usual doses might have been dangerous. Antiplasmin levels should be determined only in patients with clinical and laboratory evidence of severe hepatic failure. I do not think that measurement of fibrinolytic activity during therapy is required. We have often obtained therapeutic effect in patients and experimental animals without having generated a free fibrinolytic state in plasma.

This point is best illustrated by the following experiment (Table II): I¹³¹-labeled human fibrin clots were prepared in the large vein of dogs. Without treatment or on infusion of AP alone, decrease in radioactivity was less than 13 per cent in twenty-four hours. Fifteen RPMI units of human SK-plasmin per kg. resulted in about 60 per cent lysis in twenty-four hours. When the same amount of SK, as contained in this preparation, is infused alone only 28 per cent decrease in radioactivity occurs in twenty-four hours. This is due to the fact that SK has very little activating effect on the fibrinolytic

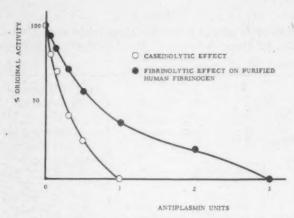


Fig. 4. Comparison of caseinolytic and fibrinolytic activity of three units streptokinase-activated plasmin mixed with various amounts of human antiplasmin.

system of dogs and endogenous activation of human clots apparently plays a small role under these conditions. When the same amount of plasmin is neutralized with AP, so that the resulting preparation has no caseinolytic activity and does not activate the euglobulin fraction of dog plasma, 60 per cent lysis is still obtained *in vivo*. This phenomenon can probably be explained by the following *in vitro* experiment (Fig. 4):

Mixing a standard amount of SK-activated human plasmin with increasing amounts of AP, caseinolytic activity rapidly decreases while fibrinolytic activity decreases more slowly. In mixtures which show no caseinolytic activity, about one-third of the fibrinolytic activity still remains. When I131-labeled plasmin is infused in vivo into dogs, localization of radioactivity on fibrin clots can be demonstrated.7 From these experiments it seems that fibrin can successfully compete for plasmin with AP. Thus, when plasmin is infused at a slow rate, rapid formation of a complex with the large reservoir of AP in normal plasma may prevent the appearance of free plasmin activity in the blood. Yet the fibrin clot may competitively take up sufficient plasmin during its long exposure to the circulating complex to cause clot lysis. I believe this mechanism may explain the large number of favorable results both in patients and in experimental animals when no free plasmin could be demonstrated during infusion.

All determinations of fibrinolytic activity in blood are too time consuming to be of practical value in clinical therapy. For practical purposes it is sufficient to perform periodic Lee-White clotting time determinations and to observe the blood tubes at room temperature for

spontaneous lysis. Increases in clotting time beyond two to three times normal and/or spontaneous lysis of the clot in less than one hour should be an indication for slowing down the infusion.

SUMMARY

DR. BAUMGARTEN: In summarizing this discussion, it is apparent that standardization of fibrinolytic agents should be carried out by measuring the lysis of a fibrin clot. Most investigators agree on this. However, in addition, other types of assays may well be considered as adjuncts. Breakdown of an assay into proteolytic and activator activities can be carried out by employing casein as the substrate of choice. Some investigators prefer heated and unheated fibrin plates for such differential assays. It is a matter of preference of the investigator as to which assay procedure is employed. However, for purposes of standardization, one method should be stipulated as the primary assay. I recommend casein as the substrate of choice, as the results obtained with it are more reproducible than those obtained with the fibrin plate. Apparently casein has been used extensively in many laboratories throughout the world. Thus, while the clot lysis test assays the sum of proteolytic and activator activity, individual assay of the proteolytic and activator activity can be achieved with casein substrate.

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Intravascular Clotting

A Biologic Error*

WILLIAM T. FOLEY, M.D. New York, New York

THE CHANGE of blood from a liquid to a solid state is nature's method of protection against blood loss. This phenomenon is activated in a non-specific way by a number of clinical conditions that lead to platelet deposition and release of thrombogenic substances. Evolution has not yet devised an effective means of completely and quickly combating this clotting of blood when it is an error; that is, detrimental to the organism. The clinical states that cause this undesired physical metamorphosis of blood will be discussed and case reports will be presented.

CLINICAL DISORDERS WITH INTRAVASCULAR CLOTTING

PHLEBOLITHS

The change of blood from the fluid to the solid state is a protective mechanism against hemorrhage which might result from trauma. This process occurs inside blood vessels in all persons, in health and in disease, as is easily demonstrated on the x-ray film of the pelvis of any adult. Phleboliths are noted as small white spots of calcification scattered about the pelvis. Having identified them as small bits of calcification in the veins, we are generally told that they are found in all persons and are of no clinical significance. Although these spots of calcium represent areas of thrombosis in veins, we know little more about them.

SURGERY

All surgery produces thrombosis in blood vessels. Each time a surgeon cuts the skin, hemostats are placed on the severed vessels. The vessels are traumatized and a clot is produced. In the pelvis large veins are often

damaged and extensive phlebitis results. Pulmonary emboli can occur from these clotted veins as in the following patient:

CASE 1. Pulmonary Embolism from Surgery: A house-wife, age sixty-five, was found to have a carcinoma of the endometrium. Operation was performed on January 28, 1957, and consisted of total removal of the uterus, tubes, ovaries and lymph nodes in the iliac and obturator areas. Pathologic diagnosis was found to be adenoacanthoma with slight to moderate endometrial invasion and vascular lymphatic involvement.

Postoperative Course: The patient had a calm postoperative course during the first twelve days. Her temperature remained between 37 and 38°c. She was allowed up to the bathroom, had her meals sitting up and walked about the room. On February 10, eleven days after the operation, she complained of pain in the right lower part of the chest. The pain was made worse by lying on the right side. Deep breathing was impossible. Examination showed splinting of the chest on the right side. Moist rales were present at the posterior base on the right side. Her temperature rose to 39°c. Examination of the legs was completely normal. The white blood count was 7,800 per cu. mm.; the differential count showed 69 mature polymorphonuclear leukocytes, 10 band cells, 12 lymphocytes, 8 monocytes, and 1

Roentgenograms of the chest showed a localized area of infiltration in the right posterior costophrenic sulcus, with a small amount of pleural reaction around it (Figs. 1 and 2).

In the differential diagnosis, the conditions to be considered were pneumonitis and pulmonary embolus. The time of onset of pleuritic pain and the response to therapy made the diagnosis of pulmonary embolus likely. During any extensive surgery in the pelvis, a great many small veins have to be tied off. These veins thrombose and thus represent potential sources of emboli. Whether or not this is of clinical signifi-

^{*} From The Cornell University Medical College and the Vascular Clinic, The New York Hospital, New York, New York.

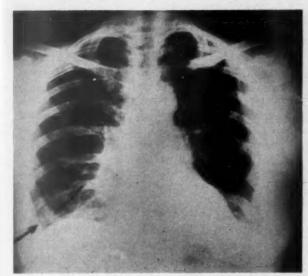


Fig. 1. Pulmonary embolism from surgery. (Figures 1 through 4 from: Foley, W. T. and Wright, I. S. Colored Atlas and Management of Vascular Disease. New York, 1959. Appleton-Century-Crofts, Inc.)

cance depends on whether or not a clinically detectable embolus actually does occur.

Treatment: The only therapy consisted of heparin (concentrated to 20,000 units per ml.) which was given subcutaneously every twelve hours; an initial dose of 15,000 units was given. Blood coagulation tests were carried out daily. The aim was to obtain a maximum of three times the control coagulation time in a period of four hours after administration of the heparin. Coagulation time should return approximately to normal by the twelfth hour. The dose was varied each day, ranging from a low of 10,000 units to a high of 15,000 units. This was continued for a period of three weeks. The fever slowly subsided during this period. Symptoms referable to the chest persisted for about four days and then gradually regressed. Rales persisted for twelve days. The abdomen was tender, but not more so than would be expected after such a major surgical procedure. The legs were clinically normal at all times. Because of the absence of involvement in the legs, it was not necessary for this patient to wear elastic stockings.

STASIS

Stagnant blood tends to clot. Civilization has developed many customs which lead to stagnation of blood. Modern clothing is not designed for proper hygiene. Men wear circular garters that act as a tourniquet on the flow of venous blood in the legs. The girdles worn by women are designed for standing; none are designed for the woman when she sits down and, if she sits for many hours at a time, phlebitis can follow.

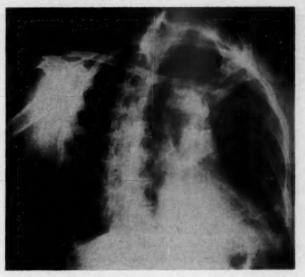


Fig. 2. Pulmonary embolism from surgery (oblique view).

CASE 2. Thrombophlebitis Due to Stasis: A middleaged woman gave a history of varicose veins being prevalent in both sides of her family. She inherited weak venous valves. After years of standing, these valves ruptured successively until the entire venous system from heart to ankles was devoid of this protection. Under this increased head of pressure, the superficial veins became widely dilated and varicose.

On a long automobile trip she wore a girdle. In the sitting position, this girdle bunched up in the groin and acted as a tourniquet to impede blood flow. The stagnant blood clotted in the veins. The following day she noticed red, tender, hot cords along her inner thigh. They quickly extended down to the knee and calf. The following day the entire leg was swollen due to blockage of the deep veins. A fever developed.

On examination, the long saphenous vein was found to be thrombosed from mid-thigh to calf. Large clots filled the dilated varices. Some measured as much as one inch in diameter. The foot and ankle were swollen with 3 plus pitting edema. The calf at its maximum circumference measured two inches greater than the other calf. On dependency, a deep cyanosis developed. When standing, collateral veins became prominent over the hip. Weight-bearing produced pain in the foot and leg. Passive flexion of the foot elicited pain in the calf.

Therapy: She was placed at bed rest and the foot of the bed was elevated on six-inch blocks. Hot moist packs were applied. Anticoagulants were administered; 15,000 units of heparin were given subcutaneously every twelve hours. The concentrated form was used (10,000 units per ml.). On the third day Dicumarol® administration was started. Therapeutic levels of prothrombin time were reached on the fifth day. Heparin administration was discontinued and Dicumarol was maintained at a daily

dose that kept the prothrombin time between twenty-five and thirty-five seconds.

Pain and tenderness subsided rapidly, but the hard clots persisted for many weeks. On the tenth day, her temperature returned to normal and she was allowed to walk. A well fitted elastic stocking was made. It extended from the toes to one inch below the knee. On the twentieth day she was discharged from the hospital.

After Care: She was instructed to (1) walk about with the stocking on; (2) sleep with the foot of the bed elevated; (3) swim or walk in deep water as often as possible; (4) elevate the feet on a footstool when sitting; and (5) avoid tight garments and sitting for long periods of time, as in a train or plane trip.

Dicumarol administration was continued for an additional six weeks. Each week her prothrombin time was checked, and she was cautioned to expect aches and pains in her legs from time to time, especially when there was a sudden fall in barometric pressure. After six weeks, the dose of Dicumarol was gradually decreased, then stopped two weeks later.

She returned to her position as a clerk. One year later, her leg showed the varicose veins as before, but no additional signs of venous insufficiency had developed, as her elastic stocking protected her. She was told that her venous insufficiency could be improved only by surgery but that, if she followed the regimen outlined, she might avoid further difficulty.

Stasis may also occur from disease. Patients with congestive heart failure have sluggish blood flow. In addition, they are apt to be sedentary or to lie in bed for long periods of time. For these reasons, thromboembolism is the major complication of heart disease. In patients confined to bed because of any illness phlebitis is prone to develop. Patients with arthritis or senile patients often sit all day in a chair without moving. Thrombosis in situ in the tissues is apt to occur. Swelling, ulcerations and cellulitis follow.

EDDY CURRENTS

In areas where the blood flow forms peculiar eddy currents, such as in the auricle of the heart when the mitral valve is deformed, or in the wall of an artery that has an aneurysmal dilatation, thrombi tend to form. This is why emboli are so common in rheumatic heart disease. Inflammation or damage to the wall of a blood vessel or to the wall of the heart gives rise to thrombus formation. This is demonstrated by mural thrombi which form over a myocardial infarct. The thrombi may break off and produce emboli in large and important vessels as in the following patient.

Case 3. Arterial Embolism: This fifty-one year old woman, a factory worker, had been well until November 1954, when severe angina pectoris developed. This gradually increased and after six weeks it culminated in an episode of severe substernal pressure, which led to admission in another hospital. A diagnosis of myocardial infarction was established. Anticoagulants were not given. On the fourteenth day, while straining on a bedpan, a sudden pain developed in her left leg. The leg rapidly became cold and blue. She was then transferred to our hospital.

Examination showed cold, pulseless, cyanotic legs. Femoral pulses were absent. Our diagnosis was embolization of the lower abdominal aorta from a mural thrombus secondary to myocardial infarction.

Treatment consisted of anticoagulant therapy and the use of an oscillating bed and reflex heat. The legs became warmer, but the left large toe became black and mummified. Collateral flow developed down into the foot. All areas became pink and warm except for the black toe. The patient became ambulatory and was discharged. In March (four months after her embolic phenomenon), the necrotic toe was gently twisted off with a thumb forceps. The patient was encouraged to walk long distances slowly. Arterial flow continued to improve and perfect healing was obtained.

A follow up for three and a half years shows continuous improvement in collateral circulation. She has resumed work at her former job.

If such a patient is seen within a few hours after embolism blocking the aorta, iliac or femoral arteries of the lower extremities or the subclavian-axillary arteries, serious consideration should be given to immediate embolectomy. The general condition of the patient must be considered before such an operation. A patient who has a recent myocardial infarction is usually considered a grave surgical risk.

ARTERITIS

Any disease which gives rise to an inflammation in the wall of a vessel can produce blood clotting at that site. Two examples of this are in the following case reports.

Case 4. Ergot Poisoning: A middle-aged building superintendent had suffered from migraine headaches for many years. He had polycythemia. He had taken ergotamine tartrate at frequent intervals which had affected his headaches favorably. For two weeks prior to admission, he had taken unusually large doses. Gangrene developed in the right fourth toe. The foot was cold; the vasospasm was severe.

Treatment consisted of withdrawal of the ergotamine, reflex heat applied to the groin, the use of an oscillating bed and walking for increasing distances hourly during the day. Healing took place without difficulty.



Fig. 3. Gangrene of the hands from tobacco idiosyncrasy.



Fig. 4. Healing after tobacco interdiction.

CASE 5. Nicotine Idiosyncrasy: Before we first saw him, a physician in early middle age had had three attacks of superficial phlebitis. Spasmodic blanching of the fingers and toes after slight chilling of the body then developed. Ulcerations formed on the finger tips. Examination showed sluggish radial and occluded ulnar pulses. He was hospitalized. He had been accustomed to smoking twenty cigarettes daily but stopped this habit completely during the first hospital stay.

Treatment: He was given fever therapy in the form of intravenously administered typhoid vaccine. Using a dilution of 100 million organisms per ml., five million bacteria were given the first day. Four hours later he had a slight chill, followed by a temperature of 101°F. We prefer to obtain two to three degrees of fever without a chill, but this is not always possible. The temperature remained elevated for two days. On repetition of the treatment no elevated temperature was produced. The dose was, therefore, increased by three million organisms every fourth day. Healing was well advanced in three weeks, at which time he was discharged.

He returned to a busy general practice. For six months he succeeded in avoiding tobacco. However, during a period of great stress, he resumed smoking. The disease promptly became active and new ulcers appeared. Figure 3 shows the gangrenous hands. The radial, ulnar, dorsalis pedis and posterior tibial vessels were occluded. Because of the intense pain,

he had become addicted to narcotics.

He was again admitted to the hospital. With great difficulty he gave up smoking. Narcotic doses were reduced, then successfully omitted. Again fever therapy was given. Collateral flow developed and good healing was obtained with only minor loss of tissue (Fig. 4).

If this patient should resume the use of tobacco, the disease may be expected to involve other vessels, such as the brachial, iliac, coronary, cerebral or mesenteric arteries.

DIABETES MELLITUS

This disease notoriously gives rise to a rapid type of atherosclerosis. Involved in diabetes are many complex factors which are not understood. There is a vasculitis that seems to be independent of the defect in carbohydrate metabolism. In patients in whom the glucose metabolism is well controlled by diet and insulin, extensive vasculitis develops nevertheless. Diabetes affects not only the large vessels, but also involves the small vessels, such as the digital arteries. Gangrene of the toes can occur even in the presence of good pulsations in the dorsalis pedis and posterior tibial arteries.

CASE 6. Diabetic Gangrene: A fifty-eight year old municipal worker presented with gangrenous toes. His other leg had been amputated one year previously at another clinic. He was able to walk well with his artificial leg but had taken to bed when his toes caused pain. He had an occlusion of his femoral artery in Hunter's canal.

Therapy: He was treated as an ambulant patient and not admitted to the hospital. The diabetes was controlled by administration of tolbutamide and by diet. Each hour he walked for a minimum of five minutes. He gave up the use of tobacco. Furacin soluble dressing was applied daily to his toes. The necrotic toe separated at a line of demarcation. The bone was severed with scissors. He was encouraged to walk and reported for weekly debridement. Com-

plete healing was obtained.

OTHER TYPES OF THROMBOEMBOLISM

Atherosclerosis: Atherosclerosis, giving rise to thrombosis, will develop in any blood vessel that has been damaged. For example, a congenital lesion such as coarctation of the aorta leads to atherosclerosis. Syphilitic involvement of the arteries, in turn, leads to

atherosclerosis, often of a severe degree. Atheromatous patches may develop at any place in the arterial system and there is always danger that thrombi will form over these areas.

Blood Dyscrasias: Polycythemia notoriously gives rise to thrombi. It produces disturbance in the clotting mechanism so that thrombi and hemorrhage can occur at the same time. All types of leukemia are associated with thromboembolism.

Toxicity: Toxic chemicals give rise to purpura and thromboses at the same time.

CASE 7. Phlebitis and Purpura from Drug Toxicity: This patient is an elderly woman who complained of pains in her joints. A physician prescribed phenylbutazone, 100 mg., to be taken after each meal for a period of seven days. She obtained a great deal of relief from this medication and decided to take it without consulting her physician. She continued taking the drug for a period of six months. Suddenly bleeding in her mouth, legs and thighs developed.

Examination disclosed that the bone marrow had ceased to produce granulocytic cells. There was also a complete absence of platelets in peripheral blood smears. Phlebitis had developed in the veins of the legs, as well as large hematomas and widespread ecchymoses. Bleeding had also occurred in the lips, in the oral cavity and about the nose.

Malignancy: Most types of carcinoma are associated, sooner or later, with extensive intravascular clotting. It is well known that some types of carcinoma produce a derangement in the blood clotting mechanism, leading to formation of thrombi in the heart, arteries and veins.

CASE 8. Thromboembolism from Carcinoma of the Breast: The patient was a twenty-six year old housewife who had an adenocarcinoma of the breast. Phlebitis developed which was not controllable by oral anticoagulants, and other areas of thrombosis occurred. Pulmonary emboli developed. With the attacks of migratory phlebitis, she had episodes of severe vasospasm. At autopsy extensive intravascular clotting was demonstrated.

Embolism of Unknown Origin: Sometimes extensive pulmonary emboli occur without evidence of the original site from which they came.

CASE 9. Embolism of Unknown Origin: A fifty-year old housewife had signs of progressively severe pulmonary hypertension. Clinically, she was thought to have congenital heart disease. She did not respond to therapy and died.

At autopsy, massive old and recent pulmonary thromboemboli were found. There was extensive sclerosis of the large and small arteries and arterioles in the lungs. The right ventricle of the heart was hypertrophied and dilated. No congenital abnormalities were found in the heart. The changes in the pulmonary vessels apparently were the result of repeated old and recent embolization with subsequent thrombosis. The site of the origin of the emboli was not demonstrated.

CONCLUSION

The blood clotting process is a non-specific phenomenon that can be activated by a great number of biologic disturbances. These disturbances may be secondary to activity of micro-organisms; to errors in hygiene, such as stasis and tight garments; to malignancy; and to other diseases, many of which are of unknown cause. Decade by decade the threat of intravascular clotting increases until the geriatric ages are reached, when thrombi become the leading cause of disability and death.

Evolution has not developed a mechanism for combating this reaction to errors in hygiene and to disease. Man must devise artificial means of coping with the problem. Anticoagulant drugs and lytic enzymes represent his beginning attempts to meet this problem.

DISCUSSION OF PAPER BY DR. FOLEY

DR. THEODORE H. SPAET (New York, New York): One of the things that has disturbed coagulationists is the fact that in blood everything necessary for clotting is present in intimate mixture yet the blood stays fluid. This would not be so surprising under normal circumstances but the preservation of fluidity is stubbornly maintained despite the fact that highly coagulant materials can be introduced into the general circulation. There is virtually never, except under the most unusual circumstances, the production of in vivo clotting. In the past, the explanation for this phenomenon has largely concerned circulating materials which inhibit clot formation and materials which destroy small clots if they do tend to form. However, a vast amount of coagulant material can be introduced into the general circulation with impunity. The degree of "hemostatic homeostasis" appears to exceed the capacity of circulating agents. We can perhaps formulate a law that blood will clot only if it fails to circulate. We have evidence that blood stays fluid when it circulates because coagulant activity which develops or is introduced is cleared by a cellular mechanism. Our most significant data concern fully formed blood thromboplastin. We have some rather compelling evidence that blood thromboplastin is particulate in nature and is selectively cleared by the reticulcendotheilal system.

Briefly: (1) If blood thromboplastin tagged with I131 or P32 is given intravenously it distributes itself like carbon in the rat. (2) If blood thromboplastin is given intravenously during the course of a carbon clearance, the clearance is markedly inhibited. (3) If blood thromboplastin is exposed directly to the reticuloendothelial system (for example, by injection into the hepatic circulation), material that is highly thromboplastic and would ordinarily defibrinate and kill an animal will be less toxic and will cause considerably less defibrination. On the basis of this type of finding it now seems possible that each blood coagulation intermediate is removed by a specific type of clearance mechanism. Perhaps the reticuloendothelial system may prove to be the effective mechanism for all intermediates. However, at the present time we can only speak firmly with respect to blood thromboplastin.

DR. GEORGE R. FEARNLEY (Gloucestershire, England): I hesitate to criticize any of this presentation, and as one who smokes I can accept the results of what may happen to smokers. But as a rheumatologist, even at a meeting on fibrinolysis, I cannot accept the statement that the arteritis of rheumatoid arthritis is due to steroid therapy. In England where steroids are used less, we see these complications in patients who have never received steroids. The general opinion in England is that the arteritis of rheumatoid arthritis is not caused by steroid therapy.

DR. WILLIAM T. FOLEY (closing): In the specific case of rheumatoid arthritis referred to by Dr. Fearnley, the young man recovered when steroid therapy was discontinued, suggesting that the steroids were involved in the arteritis.



Clinical Pharmacology of Various Types of Fibrinolytic Enzyme Preparations*

J. L. Ambrus, M.D., Ph.D., C. M. Ambrus, M.D., Ph.D., J. E. Sokal, M.D., G. Markus, M.D., Ph.D., †
N. Back, D.Sc., L. Stutzman, M.D., D. Razis, M.D., ‡ C. A. Ross, M.D., B. H. Smith, M.D.,
A. C. Rekate, M.D., G. L. Collins, M.D., D. L. Kline, Ph.D. and J. B. Fishman, Ph.D.

Buffalo, New York and New Haven, Connecticut

Many reports are available on the therapeu-tic effectiveness of fibrinolytic enzymes in experimental thrombosis of laboratory animals1-10 and in clinical thromboembolism.7,11-25 Some investigators have used activators of the fibrinolysin system, while others employed preformed fibrinolytic enzymes (plasmin). The most frequently used preparations were streptokinase (SK) of various degrees of purity and SK-activated human plasmin. As far as we know this is the first clinical report of therapeutic results with urokinase (UK) and UKactivated human plasmin. We have also used SK-activated human plasmin preparations in which SK was inactivated by various procedures, spontaneously-activated human plasmin and chloroform-activated bovine plasmin. In contrast to the other preparations, the last three had fibrinolytic activity without plasminogen activator effect.

Recent studies^{26,27} indicate that when SK is mixed with human plasminogen a series of enzymes are produced which transform into each other $(\alpha, \beta \text{ and } \gamma \text{ plasmin})$ and which have different biochemical characteristics. Thus, in the infusion bottles used in clinical therapy, there is a constantly changing mixture of fibrinolytic enzymes.

It appeared important to compare the various types of fibrinolytic enzyme preparations and select the most promising ones for more extensive clinical trials. Animal experiments cannot be relied upon entirely for such comparisons since the fibrinolysin systems of the various animal species differ considerably; e.g., SK

is a potent activator of human plasminogen, while plasminogen of laboratory animals is not activated or only slightly activated, depending on species and the batch of SK used.

MATERIAL AND METHODS

ASSAY OF ENZYME PREPARATIONS

Plasmin Activity: Dilution series are prepared of the material to be assayed. Of each dilution, 0.2 ml. is incorporated into a clot formed with 0.3 ml. of a 0.6 per cent purified human fibrinogen solution and 0.1 ml. purified bovine thrombin solution (containing 1 NIH unit) in imidazole buffer at pH 7.2 in a 45°c. water bath. End point of lysis is when the air bubbles trapped in the clot suddenly rise to the surface. One RPMI unit is defined as the fibrinolytic activity which dissolves the standard clot under the described conditions in two minutes. Lysis times are converted to units on a standard curve.

It is essential that both fibrinogen and thrombin should be purified from contaminating plasminogen, since, depending on the degree of contamination, lysis will indicate to a greater or smaller extent, plasminogen activator activity, rather than plasmin (fibrinolytic)activity. Differences in plasminogen contamination of the reagents used may account for some of the contradictions in the literature of this field. Human fibrinogen can be purified satisfactorily by repeating the procedure of Laki²⁸ three times. Bovine thrombin is purified by repeated alcohol precipitation or by treatment with β-mercaptoethylamine.²⁹

The test for plasminogen contamination of the reagents is based on the following: When increasing concentrations of SK are added to a standard unitage of human plasminogen, the amount of plasmin generated first increases with increasing concentra-

^{*} From the Roswell Park Memorial Institute, The University of Buffalo Medical and Graduate Schools, The Edward J. Meyer Memorial Hospital, Buffalo, New York and Yale University Medical School, New Haven, Connecticut. This work was supported by grants from The American Heart Association, The National Heart Institute, U. S. Public Health Service and The American Red Cross Blood Program.

[†] Established Investigator, American Heart Association. ‡ Clinical Research Fellow, U. S. Public Health Service.

tions of SK, reaches a maximum, then decreases. The amount of SK required to produce maximal activation depends on the concentration of plasminogen. In order to test for plasminogen contamination, a series of SK concentrations are incorporated into standard clots and observed for lysis as described. The shortest lysis time obtained will represent the optimal activating concentrations of SK for the amount of plasminogen present in the clot. The quantity of contaminating plasminogen can be estimated from standard curves. Even grossly contaminated clots will show no lysis when tested with high (inhibitory) concentrations of SK. With the standard clot used in these studies the shortest lysis times were between ten and twenty hours and were obtained with about 10 units of SK per clot. According to our definition, 1 RPMI unit of plasmin corresponds to two minute lysis. The longest lysis time which can be expressed quantitatively on our standard curve is two and a half hours, corresponding to 0.01 RPMI units of plasminogen. Most plasmin assays are completed within one hour. A ten-hour lysis time represents a negligible amount of plasminogen contamination, which cannot be expressed in units.

Activator Activity: In addition to their plasmin activity, all preparations used were tested for their ability to convert human plasminogen to plasmin. This was termed activator activity. The test is performed by preparing a dilution series of the preparation under study and mixing each member of the series with an equal volume of a 1 per cent plasminogen solution. After ten minutes incubation at 28°C. the mixtures are assayed for plasmin activity on the standard clot at 45°C., as already described. The increase in plasmin activity as compared to the activity of the preparation alone is the activator activity. One RPMI unit of activator is the activity which produces 1 unit of plasmin from 1 unit of plasminogen under the conditions described.

SK Activity is expressed in Christensen units. 45
UK Activity is expressed in Plough units. 36

ASSAY OF FIBRINOLYTIC FACTORS IN THE CIRCULATION OF PATIENTS

Plasmin activity is determined in plasma and in the euglobulin fraction of plasma. Two-tenths of a ml. of undiluted plasma and of 1:2 and 1:4 dilutions are incorporated into standard clots and lysis observed at 28°c. and 45°c. The euglobulin fraction is assayed undiluted at both temperatures. Lysis times longer than three hours cannot be converted to units on our standard curve and are referred to as "trace" fibrinolytic activity. Details of the assay are identical with that described under assay of enzyme preparations.

Plasminogen assay is performed on serum and euglobulin fractions of plasma. Both are incubated with an equal volume of an SK dilution series for ten minutes at 28°c. and assayed for plasmin activity by incorporating 0.2 ml. of the mixtures into the standard clot at 45°c. The fastest lysis time is obtained with the mixture having the optimal SK-plasminogen ratio. This lysis time converted to units represents plasminogen content per 0.1 ml. of serum or euglobulin fraction.

Activator Activity: Serum or euglobulin fractions are incubated at 28°c. for sixty minutes with equal volumes of a 1 per cent human plasminogen solution. Plasmin assay of the mixtures is performed on the standard clot at 45°c. as previously described. Increase in plasmin activity, as compared to the activities of serum, euglobulin and plasminogen solutions alone, represents the activator activity of serum or euglobulin fraction. Each RPMI unit of plasmin generated corresponds to 1 RPMI unit of activator activity.

Antiplasmin Activity: Serum is incubated with an excess amount of spontaneously activated plasmin³⁰ (devoid of activator activity) for sixty minutes.

Plasmin incubated under the same conditions serves as a control. Both mixtures are assayed for plasmin activity at 45°c. as described earlier. Decrease of plasmin activity during the period of incubation (after correction for the control) corresponds to the amount of antiplasmin present in the serum. One RPMI unit of antiplasmin is defined as the amount which will inhibit 1 RPMI unit of plasmin.

ANALYSIS OF BLOOD CLOTTING FACTORS

Studies on blood coagulation included the following analyses: (1) modified thromboplastin generation test, 31 (2) one-stage prothrombin time, 32 (3) factor v, 32 (4) factor v1183 and (5) fibrinogen. The Lee-White coagulation time, bleeding time and platelet count were determined by routine clinical methods.

ENZYME PREPARATIONS

Plasminogen is prepared from Cohn's fraction III of human plasma (obtained from the American Red Cross Blood Program) according to the method of Kline34 under aseptic conditions. A 1 per cent solution is made up in 0.1 M NaHCO₃ buffer at pH 8.5, filtered through bacteria-retaining filters and lyophilized or stored in the frozen state. Each batch is tested for fibrinolytic and activator activity after mixing with a wide range of SK and UK concentrations. SK and UK: plasminogen ratios are selected which produce optimal fibrinolytic and activator activities (the two are usually closely related). Corresponding amounts of SK and UK are used for activating plasminogen immediately before treatment. Activation is performed at 28°c. and pH 8.5. Purified SK for activating plasminogen, or for therapeutic use alone, was obtained from Merck, Sharp and Dohme Co. UK was obtained from the Leo Co. (Copenhagen, Denmark) or prepared by modifications of the methods of von Kaulla⁸⁵ and Plough and Kjeldgaard. 36 In certain instances

SK-activated human plasminogen was further purified by the method of Fishman and Kline.³⁷ A series of preparations were made in which the latter material was treated with acid and/or alkali and/or with further precipitation procedures7,38 in order to denature or remove SK and activator activity. These preparations are able to dissolve fibrin clots in vitro but do not activate plasminogen. An additional preparation of SK-activated human plasminogen was obtained from Merck, Sharp & Dohme Co. (Thrombolysin®). This preparation is similar to that prepared by ourselves, except that plasminogen was prepared from human plasma by the alcohol fractionation method of Cohn (fraction III) and the SK added was somewhat more than considered optimal by us. UK-activated human plasminogen made by methods identical to ours was also obtained from Parke, Davis & Co. Chloroform-activated bovine plasmin was prepared by the method of Loomis³⁹ and supplied by Parke, Davis & Co. Other preparations used by our group^{7,38} were given only to a few patients, often only on one day of a treatment schedule, and are therefore not included in this study.

All solutions were freshly made up before treatment. With a few exceptions, 5 per cent dextrose in distilled water was used as solvent. In patients with diabetes, physiologic saline was used as solvent. Usually the total volume was 500 cc. for eight-hour infusions and 250 cc. for four-hour infusions. The treatment schedule used most often consisted of a 30 RPMI unit/kg. infusion lasting eight hours, on the first day, and two 15 RPMI unit/kg. infusions lasting four hours each, during the subsequent two days. All preparations were found to be unstable at room temperature; some lost as much as 25 per cent activity in fifteen minutes. At 4°c. even the most unstable preparations showed less than 10 per cent decrease in activity at the end of eight hours. For this reason, double walled plastic jackets were constructed into which standard infusion bottles could be placed and hung on infusion stands. The outside surface of the jackets was covered with insulating material and the space between the two walls filled with refrigerant. The latter froze within two hours when placed into a deepfreezer and was able to maintain temperatures of 2° to 4°c. in the infusion bottle for four hours at a room temperature of 22°c. At this time the jacket was exchanged for one freshly removed from the deepfreezer. Higher outside temperatures required more frequent change of the jacket. On warm days, the jackets were exchanged every two hours. Temperatures of the infusion fluid actually reaching the patient (as measured at the distal end of the infusion set while the infusion was running at usual rates) varied between 19 and 21°c.

CLINICAL METHODS

The chief purpose of this report is to compare

various types of fibrinolytic enzyme preparations and to analyze side effects. The largest group of patients treated, in whom most of the different types of preparations under study were tested, and in whom objective methods of evaluation are available, is the one containing 118 cases of thrombophlebitis and thrombosis of large veins. Therapeutic results in other disorders will be reported elsewhere.40 Methods of evaluation of therapeutic results in thrombophlebitis have been described in detail previously and pitfalls have been discussed.14 Objective clinical methods of evaluation included: measurement of circumference of the affected limb, Lowenberg's cuff test,41,42 Homans' sign, measurement of skin temperature, infrared photography and phlebography; in a few cases of thrombosis of the large veins, venous pressure was measured by the direct method. The condition of the patient often did not allow performing certain measurements and in some cases other considerations led us to proceed with treatment before all base line measurements could be obtained.

The shortest time in which improvement was observed in untreated control subjects was six days from establishment of diagnosis. Accordingly, we considered as "improved" only those patients treated in whom objective signs of improvement were obtained within five days from the first treatment. For purposes of tabulation, improvement was designated "complete" if all objective and subjective signs returned to normal and "partial" if only some returned to normal or if all showed decrease in severity but none disappeared entirely.

The majority of patients in this group were elderly and bedridden; many suffered from neoplastic diseases. This explains the relatively high rate of secondary pulmonary embolism in the untreated or anticoagulant-treated groups. Anticoagulant therapy usually consisted of heparin followed by dicumarol. Patients who were treated with fibrinolytic enzymes were given heparin about two hours after completion of the first enzyme infusion. This time was selected since it was shown previously43 that free plasmin disappears from the circulation within two hours after infusion. Heparin was discontinued and the next day's infusion withheld until Lee-White coagulation time returned to the normal range. Two hours after completing the infusion, the patients were again given heparin.

This was not a double blind study. No matched cases were employed. Patients in the control group were so classified for the following reasons: (1) Fibrinolytic enzyme preparation suitable for clinical use was not available when the patient was admitted. (2) In consultation on another service we expressed willingness to accept the patient for the study. The physician in charge, however, did not want to subject the patient to treatment with an experimental drug and requested that we use anticoagulants or conservative treatment.

RESULTS

SUMMARY OF PRECLINICAL STUDIES

The over-all plan of this study was to prepare different types of fibrinolytic enzymes, test them for in vitro fibrinolytic and activator activities, and study the promising ones in vivo. Over fifty preparations were eliminated in the in vitro experiments or in the early phases of study in animals. A commercial SK-activated plasmin preparation was eliminated because the number of plasmin units in each vial was so small that the minimal dose required for the treatment of thrombosis on the basis of our previous experimental and clinical studies7,14 would have represented prohibitive bulk and expense. Preparations which passed in vitro screening were used for the treatment of I131-labeled experimental fibrin clots in dogs using methods previously described.7,44 Effect on clotting factors, members of the fibrinolysin system, formed elements of blood, blood pressure, cardiac function, electrocardiogram, respiration, intestinal and uterine motility, rectal temperature and electroencephalogram were tested at the same time. Acute toxicity tests were performed in mice and dogs. Autopsy and histopathologic study was undertaken on each animal which died.7 Preparations which were considered for clinical studies on the basis of these experiments were tested in at least two monkeys by these methods, and in six rabbits for pyrogenicity by the USP procedure.

Preparations of plasmin were effective in lysing experimental clots in dogs and monkeys when given in doses of 15 RPMI units/kg. or more, for one to five days. With doses up to 30 units/kg. no important physiologic changes were observed except for decrease in the fibrinogen, prothrombin, factor v, factor vII and antiplasmin levels. Changes of the first two were more pronounced than those obtained with similar doses in man. Leukopenia followed by leukocytosis was regularly seen in dogs, while this was only occasionally seen in patients. Doses of 60 units/kg. or more given in one- to four-hour infusions often caused hypotension in dogs and monkeys. When given by rapid intravenous injection even 30 units/kg. would cause fleeting hypotension in dogs. Preparations of SK or UK alone, given in doses up to 25,000 units/kg., did not cause hypotension.

CLINICAL STUDIES

Table 1 shows the distribution of patients in

TABLE I Classification of Patients by Diagnoses

Thrombophlebitis	118 12 18
Peripheral artery occlusion	
Coronary artery occlusion	18
Cerebrovascular thrombosis	71
Pulmonary embolism	23
Retinal artery thrombosis	1
Coronary endarterectomy (preventive)	6
Thrombosis in surgical skin flap	3
Priapism	1
Intermittent claudication	2
Adhesive peritonitis	1
Empyema	3
Hyaline membrane disease	12
Cystic fibrosis of pancreas (mucoviscoidosis)	3
Inflammatory conditions, cellulitis	7
Total	281

this study by diagnosis. All are included in the consideration of side effects, but only the 118 cases of thrombophlebitis and thrombosis of the large veins will be analyzed for therapeutic results. At the present time, only in this group is comparison of different types of fibrinolytic therapy possible. Preparations which were found effective in treating thrombophlebitis of terminal cancer patients, without producing pyrogenic or other side effects, were considered for study in other thromboembolic disorders (e.g., coronary occlusion, cerebrovascular thrombosis). Despite rigorous preclinical testing, pyrogenicity was often detected only clinically. Apparently routine pyrogen tests in rabbits cannot be relied upon for these preparations.

Of the 281 patients in this series, eighty-five were control subjects (untreated or anticoagulant treated), and 196 were treated with fibrinolytic therapy. The latter patients were given a total of 538 intravenous and/or intra-arterial infusions, two intraperitoneal and three intrapleural instillations and thirty-nine aerosol treatments.

Side Effects: Table II summarizes the clinical side effects. Earlier preparations showed a high incidence of pyrogenic reactions; this became rare (about 1 per cent) with later more purified preparations. The first column summarizes all preparations, the second column only the purified preparations. Hypotension, which occurred with earlier preparations, was not seen with the more purified ones. Dia-

TABLE II
Side Effects of Fibrinolytic Therapy

	Route of Administration								
Side Effects	Intravenous o	r Intra-arterial	Aerosol	Intraperitoneal	Intrapleural				
	All Preparations (538)*	Purified Preparations (385)	Purified Preparations (39)	Purified Preparations (2)	Purified Preparations (3)				
Hypotension	10	0	0	0	0				
Chills, fever	47	4	0	0	0				
Diaphoresis	6	4	0	0	0				
dominal pain	15	4	0	0	0				
Hemorrhage	12	11 -	0	0	On				
Pulmonary embolism:	2	2	0	0	0				
Local irritation	0	0	0	0	0				

^{*} Figures in parentheses represent the number of treatments.

TABLE III

Hemorrhagic and Thromboembolic Complications of Fibrinolytic Therapy

	No.	Type of Preparations
Total infusions	538	
Hemorrhages	12	
coagulants(Apparently due to	4	2 SK-P, 2 UK-P
anticoagulants) Accentuation of pre- existing hemorrhage, oozing from neoplas-		2 UK-P
tic lesions	4	1 SK, 1 SK-P, 1 UK-P, 1 UK
Oozing from needle punctures, cut down sites	2	1 SK-P, 1 UK-P
Gastrointestinal hemorrhage, hema- turia, oozing from needle punctures,		
shockPulmonary embolism	1 2	SK SK-P
(1) Thirty minutes after initiating infusion (2) Clot older than five days, third day of treatment.	2	GR-1

phoresis, nausea, vomiting and abdominal pain were rarely observed (about 1 per cent). These side effects were seen with various preparations and were largely independent of the dose. They may represent contamination rather than the effect of the enzyme preparations proper.

The most important side effects were twelve cases of hemorrhage (eleven associated with purified preparations) and two cases of embolism developing during treatment. Table III summarizes these episodes and the types of preparations involved. Four of the hemorrhagic episodes were associated with anticoagulant therapy, and laboratory studies suggested that at least two were due to the anticoagulants rather than to fibrinolytic enzymes. episodes consisted of accentuation of preexisting hemorrhage or oozing from neoplastic lesions. In many of the treated patients, however, suffering from similar conditions, therapy did not produce hemorrhage or accentuate existing hemorrhage. Spontaneous, severe epistaxis was observed in one patient treated with SK-activated plasmin. The only serious clinical episode occurred in a patient treated with SK alone in whom gastrointestinal hemorrhage, hematuria and oozing from needle punctures developed, resulting in vascular collapse. One patient (not indicated in this table), who had chronic granulocytic leukemia and thrombosis, died overnight following therapy with SK-activated plasmin. Autopsy revealed hemorrhagic necrosis in some of his enlarged, retroperitoneal lymph nodes, but no causal relationship could be established between plasmin therapy and death.

In animal experiments, no indication was seen

TABLE IV

Decreases of Levels of Factors of the Blood Clotting and Fibrinolysin Systems by Fibrinolytic Therapy

	SK-P (20)*		SK (10)		UK-P (20)		UK (5)	
	Occurrence (%)	Mean Degree (%)						
Platelet count Thromboplastin	45	15	30	20	30	10	20	20
generation test	10	40	20	30	5	20	0	.0
Factor v	15	18	50	20	20	25	0	0
Factor VII	30	25	70	29	35	29	20	30
Prothrombin	45	16	30	13	30	27	40	25
Fibrinogen	65	20	40	27	40	20	60	25
Plasminogen	55	24	90	68	45	30	60	23
Antiplasmin	80	12	70	20	50	5	20	8

^{*} Figures in parentheses represent the number of treatments.

of fibrinolytic therapy dislodging parts of clots and producing secondary embolization. Nevertheless, this was a major source of worry before initiating clinical studies. Of all patients treated, only two cases of pulmonary embolism were recognized after initiation of therapy. One episode occurred in a patient with thrombophlebitis of the legs thirty minutes after starting the first infusion into the antecubital vein; too early for plasmin to have had any effect under these conditions. Only about 3 units/kg. of plasmin had been absorbed. The second case occurred on the third day of treatment in a patient suffering from thrombophlebitis of about one week's duration. Although clots of this age do not usually respond to fibrinolytic therapy,14 it cannot be excluded that therapy might have played a role in the development of embolization.

No side effects were seen in any of the patients treated by intraperitoneal or intrapleural instillation or by aerosols. The latter group represented infants with hyaline membrane disease or cystic fibrosis of the pancreas.

Attempts to eliminate the development of chills and fever after the administration of pyrogenic preparations by the use of chlor-promazine, Benadryl,® aminopyrine, salicylates, morphine and calcium gluconate alone or in combination were unsuccessful. The intravenous administration of Amytal Sodium® or Pentothal Sodium® in doses sufficient to produce light anesthesia, arrested even severe chills, but did not prevent the development

of hyperthermia and an increase in the basal metabolic rate.

It is well documented that pyrogens alone can produce fibrinolysis in man, presumably by liberating tissue activators into the circulation.46-52 In fact it has been questioned whether a commercial SK-plasmin preparation, which produces fever in about half of the patients treated, does not act chiefly as a pyrogen. Therapeutic results were obtained in our series with non-pyrogenic preparations. These same preparations were also effective against experimental clots in dogs and monkeys, species in which pyrogens do not produce fibrinolytic activity. We believe that the therapeutic effects obtained in this study are due to fibrinolytic activity proper and not to the side effects of the treatment.

EFFECT ON BLOOD CLOTTING AND FIBRINOLYTIC FACTORS

Table iv summarizes the frequency of changes and the degree of decrease in the more important factors of the clotting and fibrinolytic systems. Occasional increases in these factors are not tabulated since they were thought to represent variations independent of therapy. These values are based on fifty-five cases studied adequately for all the factors mentioned with determinations made immediately before treatment and immediately after the infusion was discontinued. All data in Table iv are based on four- or eight-hour infusions with 15 or 30 RPMI units of plasmin, respectively,

TABLE V

Effect of Fibrinolytic Therapy on Factors of the Blood Clotting and Fibrinolysin Systems

	Treatment Dose								
	30 units/kg. UK-P		30 units/kg. SK-P		500,000 units SK		50,000 units UK		
	Before	After	Before	After	Before	After	Before	After	
Lee-White coagulation time									
(min.)	24	21	21	44	78	27	33	30	
RPCT (sec.)	604	690	741	743	522	767	798	853	
One-stage prothrombin time									
(%)	21	23.5	27.5	17.5	27.5	17	21	19.5	
Factor v (%)	58	63	74	73	78	80	67	62	
Factor VII (% enhancement)	132	86	124	124	132	143	101	64	
Fibrinogen (mg. %)	479	458	594	493	552	490	653	637	
Thromboplastin generation				-					
test (%)	100	100	100	100	100	100	100	100	
Plasminogen (units/cc.)	6	3.6	8.8	0	6	0	7.9	6.1	
Antiplasmin (units/cc.)	9.9	9.3	10.5	9.6	11.1	10.5	12.6	11.7	
Plasmin lysis time (hr.)		12		12		12		12	

Note: Before = before infusion; after = after infusion.

TABLE VI
Effect of Plasmin Given Intravenously at Different Rates
on Factors of the Blood Clotting and Fibrinolysin Systems

		Treatment Dose						
	(three	/kg. SK-P e-minute ection)	30 units/kg. SK-P (four-hour infusion					
	Before	After	Before	After				
Lee-White coagulation								
time (min.)	12.5	22	14.3	15.5				
RPCT (sec.)	200	330	220	235 -				
One-stage prothrom-								
bin time (%)	85	60	90	95				
Factor v (%)	95	60	100	90				
Factor vn (% en-								
hancement)	145	98	130	115				
Fibrinogen (nig. %)	290	180	335	290				
Thromboplastin gen-								
eration test	100	100	100	100				
Plasminogen (units/cc.)	8.2	5.3	7.7	6				
Antiplasmin (units/cc.)	6.4	4.8	8.3	7.1				
Plasmin lysis time		65 min.		12 hr.				

Note: Before = before infusion; after = fifteen minutes after

or with 500,000 Christensen units of SK or 50,000 or 100,000 Plough units of UK. Table v shows an individual example. This patient was selected because he was treated on four successive days with SK- and UK-activated plasmin, SK alone and UK alone. Even before treatment, he suffered from prothrombinopenia and Factor v deficiency, resulting in prolonged Lee-White coagulation time and recalcified

plasma clotting time (RPCT). Dosesare indicated in Table v; infusion time was always eight hours.

Table vi shows data of a patient who was treated with one four-hour infusion of SK-activated plasmin, and on another occasion, received the same dose by rapid intravenous injection. No change in blood pressure occurred on either occasion. Decrease in clotting factors was much more pronounced after the rapid injection than after the slow infusion. When considering effects on plasma proteins, speed of administration appears to be an important variable.

It is apparent that a decrease in the levels of prothrombin, factor v, factor vII and fibrinogen may occur after standard fibrinolytic therapy. These changes, however, are never alarming and always fleeting. No dangerous changes were seen even in patients with baseline coagulation defects. Speed of infusion, however, may be an important factor and significant coagulation changes may be expected when large doses are introduced rapidly into the circulation. Changes in the coagulation system following standard fibrinolytic therapy were never equal to those occasionally seen in pathologic fibrinolytic hemorrhage. ⁵³

Plasminogen and antiplasmin levels showed significant decrease following fibrinolytic therapy in the majority of cases. In previous experi-

ments, these changes were followed for longer periods in dogs. ⁵⁴ It appeared that an initial decrease in antiplasmin level is often followed by a secondary, more persistent increase. The latter is not regular or large enough to warrant successive increase in dosage. Plasmin activity often became apparent in the plasma and more often, in its euglobulin fraction following therapy. Often, however, this could not be expressed in units since lysis time was too long to fall on the linear portion of our standard curve. In a few patients treated with SK-activated or UK-activated plasmin, no free plasmin could be demonstrated at any time, yet definite therapeutic results were obtained.

OTHER PHYSIOLOGIC EFFECTS

Leukocyte levels showed considerable variation following fibrinolytic therapy. The most common patterns were mild leukopenia followed by moderate leukocytosis (in about 30 per cent of the cases), or no change at all. No significant changes were seen in hematocrit and hemoglobin levels. No important electrocardiographic changes were seen except for transient T wave changes in patients with febrile reactions. The basal metabolic rate increased in patients exhibiting pyrogenic reactions, often before the onset of chill and hyperthermia. Serial liver function tests and urinalyses revealed no changes attributable to fibrinolytic therapy. No delayed toxicity was seen in patients observed up to three years following therapy. No serum hepatitis was reported. Autopsy and histopathologic study of those patients who died during, or shortly after, completion of therapy revealed no pathologic changes attributable to fibrinolytic therapy.

RETREATMENT

Therapeutic agents, which may find their greatest usefulness in the treatment of such frequently recurrent conditions as coronary occlusion and cerebrovascular thrombosis, may be considered for repeated use in relatively short periods of time. To explore the possible dangers of such procedures, one of us volunteered for treatment with SK and repetition of this procedure three weeks later. Two patients with cancer and recurrent thrombophlebitis were treated with SK-plasmin about three weeks after an initial course of therapy with the same agent. In the volunteer treated with SK anaphylactoid reactions developed consisting of asthma and intensive erythema over the

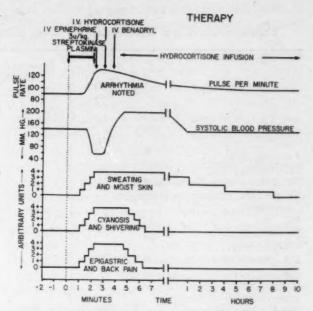


Fig. 1. Anaphylactic shock in patient with recurrent thrombophlebitis treated with SK-plasmin three weeks after initial course of therapy with the same agent.

entire surface of the skin. Recovery was gradual, over several days despite treatment with intravenously administered hydrocortisone followed by the oral administration of prednisone. One of the two patients showed no reaction; in the other anaphylactic shock developed shortly after the infusion was started. The course of his reaction is depicted in Figure 1. Treatment with intravenously administered epinephrine, Benadryl and hydrocortisone, through an infusion set inserted into a second vein as a precautionary measure, resulted in rapid recovery. This patient was then treated with UK-activated plasmin, which produced no side effects and apparently caused resolution of the thrombus. Twelve patients previously treated with UK-plasmin were retreated with the same preparation without ill effects.

THERAPEUTIC RESULTS

Table VII summarizes therapeutic results in thrombophlebitis and thrombosis of the large veins. Only patients treated within five days of the first appearance of symptoms are included in this table. It was shown previously^{7,8,14} that older clots do not respond well to fibrinolytic therapy. The dose schedule most often used was the following: the first day, 30 RPMI units/kg. of SK-, UK- or chloroform-activated plasmin in an 8-hour infusion, then for two or four consecutive days, four-hour infusions of 15 RPMI units/kg. plasmin daily. SK was given

TABLE VII
Thrombophlebitis and Thrombosis of the Large Veins Less Than Five Days Old When Treatment Initiated

Treatment	Total No.	Improved in Less Than 5 Days		Improved in More	No Improve-	Secondary Pulmonary	Died of Thrombo-
	140.	Partial	Complete	Than Five Days	ment	Embolism	embolism
None*	23	0	0	6	17	7	7
Anticoagulants*	15	1	0	8	6	5	3
SK-plasmin	16	6	8	1	1	1	1
SK-plasmin + anticoagulants.	6	3	2	1	0	0	0
SK-plasmin without activator							
activity	5	2	2	1 .	0	0	0
SK	10	1	7	1	1	0	0
SK + anticoagulants	2	0	0	1	1	0	0
UK-plasmin	11	5	4	1	1	0	0
UK-plasmin + anticoagulants.	3	0	3	0	0	0	0.
UK	4	. 1	2	0	1	0	0
Chloroform-bovine plasmin	1	1	0	0	0	0	0
Mixed fibrinolytic therapy	4	1	0	2	1	0	0
Total control subjects*	38	1	0	14	23	12	10
Total fibrinolytic therapy*	62	20	28	8	6	1	1

^{*} Controls and treated patients were not matched cases. See text.

in daily doses of 500,000 Christensen units in four- to eight-hour infusions for three to five days, UK in four-hour infusions of 50,000 to 100,000 Plough units daily for three to five consecutive days. Occasional schedules deviating from this pattern are also included, provided the minimal dose was not less than 5 RPMI units/kg. plasmin, 25,000 Plough units of UK or 350,000 units of SK. Below these doses no therapeutic effects were seen in either patients or experimental animals.⁷

Of sixty-two patients treated with fibrinolytic agents (with or without anticoagulants), in only one did secondary pulmonary embolization develop and cause death. Almost half of the group treated with fibrinolysin returned to normal in less than five days; 32 per cent showed partial improvement in this period, 13 per cent improved only after five days and 10 per cent did not improve. The therapeutic results in the group of patients treated with SK-plasmin, UK-plasmin, SK alone and UK alone were better than in the untreated group or in the control group being treated with anticoagulants. A small group of patients received SK-plasmin preparations whose activator activity had been removed. Improvement was observed in four of five cases. This indicates that activator activity is not solely, responsible for therapeutic results.

COMMENTS

It appears that several safe and potentially useful fibrinolytic enzyme preparations are now available. Which ones are most deserving of extensive clinical trials? SK and SK-activated plasmin preparations share the disadvantage of being antigenic. Our small series of three patients who were treated repeatedly and in two of whom anaphylactoid reactions developed is certainly not enough to draw conclusions as to the potential occurrence of such episodes. All three patients were challenged about three weeks after sensitization. We do not know how long hypersensitivity persists. However, skin sensitivity tests, which became positive following therapy in patients previously negative, persisted up to two years.40 With the ubiquitous presence of streptococci repeated exposures to SK is likely. SK when given alone is neutralized to some extent by pre-existing anti-SK antibodies. Accordingly, Johnson et al.20,55 and Sherry et al.56 recommended that a "priming" dose of SK calculated on the basis of the plasma volume and the antibody titer in vitro should first be given to neutralize anti-SK antibodies; then treatment with the proposed doses can be started. SK-plasmin seems to have an advantage over SK alone in that it represents preformed plasmin plus activator and is thus less subject to neutralization by anti-SK antibodies. In our

experience, when SK is used as an activator of plasmin smaller doses are needed for therapeutic results than the doses recommended by investigators using SK alone. 20,55,56 Accordingly, fewer problems of sensitization may arise in the former case. UK and UK-plasmin did not appear to be antigenic in our hands. Preparation of purified UK, however, is a more difficult and probably a less well worked out procedure than preparation of SK. Chloroform-activated bovine plasmin can probably be made most economically of all preparations, but we have assumed that it is antigenic and have not investigated it in great detail.

Sherry and associates 57 advanced the theory that fibrinolytic enzyme preparations lyse clots by activating plasminogen contaminations trapped into the meshwork of the clot, thus dissolving it "endogenously." Accordingly, they propose the use of SK alone rather than plasmin. Our group found evidence that fibrin can compete effectively with antiplasmin for plasmin and that it selectively adsorbs plasmin. 58-82 Plasmin thus continues to be present on the clot long after circulating levels have disappeared or became neutralized. This explains why, in well controlled animal experiments, lysis is first apparent only four hours after completing the infusion of plasmin, although plasmin disappears from the circulation within one hour after infusion.4.7 This also explains how fibrinolysis is possible without profound changes in circulating plasma proteins. Antiplasmin seems to play an important role in protecting plasma proteins from plasmin, yet allowing fibrin to adsorb the enzyme competitively. Experimental and clinical results obtained with preparations having no activator activity also seem to indicate that "endogenous" activation is not the only factor involved in the mechanism of in vivo fibrinolysis. While much more work is needed before the "ideal" fibrinolytic agent can be identified, preparations presently available allow us to explore the scope of clinical fibrinolytic therapy.

SUMMARY

1. One hundred ninety-six patients were treated with various types of fibrinolytic enzyme preparations.

2. Of 538 intravenous and/or intra-arterial infusions, hemorrhage occurred in twelve cases, and pulmonary embolism in two.

3. Pyrogenic side effects were seen commonly

with earlier preparations but only four times in 385 treatments with newer, more purified preparations.

4. With four- to eight-hour infusions, decrease of factors of the blood clotting system was never of sufficient magnitude to be of clinical significance. Rapid intravenous injection resulted in more pronounced changes. Factors most often affected were prothrombin, factor v, factor vii and fibrinogen.

5. Retreatment with streptokinase or streptokinase-activated plasmin three weeks after an initial course of therapy resulted in anaphylactoid reactions in two of three cases. Retreatment with urokinase-activated plasmin caused no side effects and did not result in decreased therapeutic activity in twelve cases.

6. In cases of thrombophlebitis or thrombosis of the large veins (chiefly complications of neoplastic diseases in elderly patients) of less than five days' standing when treatment was initiated, of sixty-two patients treated with streptokinase, urokinase- or chloroform-activated plasmin, streptokinase or urokinase, alone or in combination with anticoagulants, twenty-eight (45 per cent) returned to normal and twenty (32 per cent) improved in less than five days, six (10 per cent) did not improve and one died of pulmonary embolism. All preparations used appeared to be therapeutically active. The objectives and methods of this study precluded the use of a matched control group of patients on a randomized double-blind basis. Nevertheless, therapeutic results in the groups of patients treated with fibrinolysin were superior to those in the untreated group or groups treated with anticoagulants.

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DISCUSSION OF PAPER BY DR. AMBRUS ET AL.

DR. LEIF HORN (New York, New York): In discussing the toxicity of parenterally administered proteolytic enzymes and plasmin in particular, Dr. Malm and I would like to report briefly on some studies that we carried out at the Medical College of Virginia some time ago.*1 To us the results of these studies indicate that the severely traumatized animal is very sensitive to intraperitoneal injections of proteolytic enzymes. The type of injury used in our experiments was 50 per cent full thickness skin burn in adult male rats. Enzyme precursors such as trypsinogen and chymotrypsinogen, which were inactive in vitro, were also toxic in the burned rat, although completely innocuous in the non-burned animal in four times larger doses.

Rats weighing 300 gm. were burned at 240°C. in short lasting ether anesthesia. To combat shock, the rats were given intraperitoneal injections immediately after burning with 30 ml. per kg. of sterile 6 per cent dextran in isotonic saline. The freshly streptokinase (SK)-activated enzyme was prepared in our laboratories from human Cohn fraction III by the Kline procedure, and an injection was given on the three consecutive days after burn in doses corresponding to 12 mg. trypsin per kg. rat based on casein

Plasmin increased the mortality markedly. Although no deaths occurred during the twenty-four hours after the first injection, the mortality was 40 per cent following the second injection, and after the third injection the mortality climbed to 80 per cent in the rats treated with plasmin while the control

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animals still had a 5 per cent mortality at the end of the third day. Both groups received the same amount of dextran saline on the first day. The control rats were given an injection of SK, in the same dose as used for activation of the plasminogen, and crystalline bovine albumin in equal amounts on a nitrogen basis was used to compensate for the foreign protein in the plasminogen preparation.

No hemorrhage was observed during life in the rats receiving plasmin. Autopsy of the plasmin-treated rats immediately after death showed no peritoneal irritation, exudation or hemorrhage. The blood in the heart and large vessels was fluid and coagulated

rapidly in glass tubes.

The postburn course with development of a shock syndrome, viz. hypotension and hypothermia, in the rats dying from plasmin injections, was similar to that seen in burned rats given injections of trypsin, chymotrypsin, papain and a beef-spleen cathepsin C. An intraperitoneal injection of 8 mg. of trypsin per kg. on three consecutive days increased the mortality somewhat on the second and third days. An injection of 20 mg. per kg. of trypsin gave a mortality of 90 per cent at the end of the first day against 5 per cent in the control rats, and the two and three day mortalities were 95 per cent in the rats given trypsin against 20 per cent in the control rats. Chymotrypsin, given in corresponding doses, was more lethal than trypsin at the lower dose level (8 mg. per kg.) and almost identically toxic in the 20 mg. per kg. dose. Papain, given in a dose of 8 mg. per kg., did not increase the mortality on the first day, but the following injection killed all the animals in four to twelve hours during the second day. In a higher dose, 20 mg. per kg., papain killed all animals on the first day within sixteen hours.

Of considerable interest was the finding that a subcutaneous injection of trypsin in a dose of 20 mg. per kg. outside the burned area, had no effect on the mortality, but led to sloughing at the site of injection in two to three days. Unburned rats, given subcutaneous injections with this dose of trypsin, did not show necrosis or obvious signs of local inflammation. A tentative explanation for the effect of locally injected trypsin in the burned rat is that, due to the slowing down of the peripheral circulation in the burnshocked animal, the injected trypsin remained longer at the injection site than in the non-burned animal.

When an intraperitoneal injection of the inactive precursor trypsinogen was given in a dose of 20 mg. per kg. there was no increase in mortality on the first day. On the second day, however, the mortality was 60 per cent, and at the end of the third day the mortality was 80 per cent. Diisopropylfluorophosphate (DFP)-inactivated trypsin, checked by assay on several substrates, was also injected into our rat burn preparation. However, given in doses of 20 mg. per kg. such DFP-trypsin did not significantly increase the mortality.

An injection of 80 mg. of trypsinogen per kg. rat on

three consecutive days into the *unburned* control animals was completely harmless, and the rats showed no ill effects during fourteen days of observation. Likewise, an intraperitoneal injection of trypsin in doses of 20 or 40 mg. per kg. in unburned animals was harmless, while 60 mg. per kg. killed one of ten rats on the day after the third injection. Similar results were found with chymotrypsin. Papain did not produce any mortality in unburned animals in doses of 2 or 8 mg. per kg. while 20 mg. per kg. killed one of twelve rats after the third injection.

Time does not permit a discussion of the possible mechanisms underlying the toxic effects of parenterally injected proteinases. However, we wish to emphasize the possibility that the animal under severe stress or trauma may be more susceptible to adverse effects of proteinases which precipitate a lethal shock syndrome. This applies also to plasmin, although we are aware of the danger in extrapolating from findings with heterologous enzymes to situations in which proteinase from the same species is used, as is the case when human plasmin preparations are injected in man.

DR. LESLIE E. MORRIS (Pittsburgh, Pennsylvania): Dr. Ambrus, there is one figure that bothers me somewhat. In the fifteen cases of venous thrombosis, a mixture of both superficial and deep, only one patient responded to anticoagulant thereapy within five days. This does not correspond with the results that many of us encounter in practice; perhaps it might be explained by the fact that Dr. Ambrus is dealing with a hospital population where the patients are old and mostly have metastatic disease. Even so, it has been my experience that such patients initially will respond to therapy with intravenously administered heparin. On the other hand, it is a well known fact that patients with metastatic disease generally are extremely resistant to the prothrombinopenic agents.

DR. E. C. DERENZO (Pearl River, New York): Dr. Ambrus' experiments with I131-labeled preparations raise a question that I think should be considered. The problem of studying the tissue distribution and metabolism of a labeled protein is the same problem involved in working with a simple radioactivelylabeled compound such as glucose. If one is interested in determining the metabolic fate of glucose labeled with C14 one must know that C14 is present in the glucose molecule and not in a contaminant in the preparation. The problem is exactly the same when one administers an I131-labeled protein except that the problem of determining that the label is in the protein under study is more complex. It is mandatory to undertake some attempt at determining purity and homogeneity of the labeled substrate. A great deal of caution has to be exerted in the interpretation of experiments in which the metabolic fate of admittedly impure substances labeled with I131 is studied. Attempts to determine the relative abilities of radioactively labeled, impure streptokinase, plasmin or SKplasminogen mixtures to dissolve clots in vivo on the

basis of localization of radioactivity at the clot site are open to much question.

DR. JOHN S. T. Cox (Boston, Massachusetts): Dr. Ambrus mentioned that with patients there was difficulty with the phlebograms. I wondered was this social or technical, and if technical, would he like to comment on it and what his conclusions were from these technical difficulties.

DR. D. R. CELANDER (Galveston, Texas): I am very interested to learn that you have given injections of urokinase to some of these patients. If you have data on the excretion of urokinase or the patterns of excretion, could you comment on that?

DR. J. L. Ambrus (Buffalo, New York): I think Dr. Morris essentially answered his own question. The chief reason for the high incidence of secondary embolism in the control series of thrombophlebitis and the low incidence of therapeutic results in the group treated with anticoagulants alone is due to the fact that almost all patients in this series suffered from advanced neoplastic disease, most were elderly, many bedridden and cachetic. When we started this project, our colleagues who tend to be rather conservative would not refer patients to us for fibrinolytic therapy unless they were quite sure that there were not many days left. I am happy to report that in the last few years their attitudes changed considerably.

There is another criticism which applies to our series of thrombophlebitis. I think I have mentioned that this was not a double blind study. Our study in cerebrovascular accidents, coronary occlusion and hyaline membrane disease of infants is a double blind study. This leads me to answer the question of Dr. Cox. The reason phlebograms were not always obtained was not only technical, inability in finding veins in certain patients, but also such factors as the episode occurring at midnight and everybody being too sleepy and wishing to start therapy rather than arousing a grouchy roentgenologist and arguing with him that we have to have a phlebogram. Many times, we have obtained phlebograms which showed occlusion, the patient improved clinically, but then died because of his advanced neoplastic disease before we could obtain a repeat phlebogram. We might have demonstrated at autopsy that the clot is not where we would expect it to be: however, we have not considered this as sufficiently comparable evidence. There are many sources of errors in phlebography. I am sure this is not the only and ultimate

criterion on which studies on therapeutic agents in thrombophlebitis should be based. It is important to take a large number of roentgenograms in rapid sequence and to base conclusions on the whole series rather than on single frames as may be demonstrated at meetings. Vasospasm, often induced by the contrast medium, may often be responsible for the diagnosis of obstruction. Recently we have taken to incorporate papaverin into our injection of contrast medium. We have worried a great deal about the possibility of causing further thrombosis by dye injection. In many patients in whom the tendency to develop recurrent thrombotic episodes exists this was a reason for not taking phlebograms. Wherever permissible, we have heparinized the patients immediately preceeding phlebography. I believe that the dangers of arteriography are much greater than those of phlebography.

As to the question of Dr. DeRenzo, I am aware of the limitations of a study of this sort on the distribution and metabolic fate of fibrinolytic enzymes. The possibility clearly exists that some of the material labeled is not the plasmin, plasminogen, streptokinase or urokinase but a contaminating protein. We are currently combining these studies with chromatographic separation of the components and determination of specific activities as accurately as present methods permit.^{3,4}

Finally, to the question of Dr. Celander: he, together with Dr. Guest in Texas, pioneered in the urokinase field, in fact the name urokinase comes from that group. Some years ago they presented data on urokinase excretion. To my knowledge these are the only data available in the literature. We have not measured urokinase excretion following therapy.

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Review of Clinical Experience with Clot-Lysing Agents*

Eugene E. Cliffton, M.D. New York, New York

THE TREATMENT of vascular thrombosis by dissolution of the clot or thrombus has been a recent development. Although it has been recognized for years that a mechanism for fibrinolysis existed, suitable materials for clinical trial were not available until recently, and further development will be essential before we can look forward to adequate safe treatment of many difficult conditions. Even experiments on animals leading to better understanding of this lytic process have been of recent development.

Trypsin was first reported to be effective in lysis of thrombi by Innerfield et al., but subsequently the effect of this enzyme was found to be primarily antiinflammatory. Johnson and Tillett in 1952 used streptokinase to lyse thrombi produced in the veins of the ear of rabbits and were able to induce partial lysis in about 50 per cent with high doses.

In a series of experiments reported in 1954 and

1955⁴⁻⁶ it was shown that human fibrinolysin (plasminogen activated with streptokinase) could completely lyse thrombi produced in the ear veins of the rabbit and in the femoral veins and arteries of rabbits, dogs, cats and monkeys without causing significant harmful side effects. This work was confirmed using the more elaborate technic of Ambrus and his group.^{7,8} In these studies it was shown that thrombi of up to seven days' duration could be lysed, but that for practical purposes about three days was the critical time beyond which lysis would be difficult. Tillett, Johnson and McCarty⁹ were the first to report the intravenous infusion of

The first brief report on the clinical effective-

streptokinase into patients but they found the

reactions too severe to justify this as a method of

ness of fibrinolysin was made in 1956.10 The results of the treatment of twenty-seven patients with venous and arterial thrombi at various sites were reported in 1957.11 It was noted that venous thrombi and pulmonary emboli responded well but that arterial thrombi and emboli were poorly effected by the doses tolerated systemically. Local treatment was effective even with arterial thrombi. Subsequent papers12,13 and others by Moser14 and Sokal et al.15 confirmed the fact that patients with thrombophebitis responded well to treatment with fibrinolysin. The results of systemic treatment of arterial thromboses and emboli have been less convincing and in our own hands unsatisfactory until recently. In coronary and cerebral thrombosis16 a start is being made and it is hoped that some significant data will be furnished in this Symposium.

Even experimental studies on the use of fibrinolytic agents in these conditions have been limited. Ruegsegger et al.¹⁷ reported that lysis of coronary thrombi could be obtained without harmful effects and with some improvement over the control infarcts. In our own laboratory, attempts to alter the effects of internal carotid emboli were unsuccessful because of inability to obtain satisfactory controls.

Sherry and his group¹⁸ reported on nineteen patients with coronary thrombosis who had been treated by prolonged infusions of streptokinase in heavy dosage. Unfortunately they used other drugs in conjunction with streptokinase, including cortisone and antihistaminics.

There are other ways of producing fibrinolytic activity in animals and patients, including severe exercise, trauma, electric shock, cyanosis and venous obstruction, ¹⁰ and by injections of epinephrine, ²⁰ nicotinic acid, ²¹ bacterial pyro-

^{*}From the Clotting Mechanisms Section, Division of Experimental Surgery and Physiology, Sloan-Kettering Institute for Cancer Research, and the Department of Surgery, Cornell University Medical College, New York, New York. This work was supported by Grants 2867 and C 4211 from the National Institutes of Health.

Table 1
Types and Location of Thromboembolic Conditions in 197 Patients

Type and Location	No
Venous	98
Pulmonary emboli	12
Priapism	4
Arterial	36
Cerebral	10
Eye	8
Coronary	5
Induced	4
Miscellaneous	20

gens²² and urokinase.²³ Of these only pyrogens have been used as therapeutic agents.²⁴ The severe reactions obtained with pyrogens have been considered contraindications by most investigators. It is conceivable that non-pyrogenic activators can be obtained from these materials or that other activators or combinations of activators with simpler methods may be found to be most useful in the future.

Since 1958 we have been interested primarily in obtaining a material which could be infused safely in amounts to produce significant fibrinolytic activity in the bloodstream, and to maintain this activity for many hours. Two reasonably satisfactory materials are now available although they will certainly continue to be improved and better activators or enzymes may be discovered and developed. When this occurs the data already obtained can be used in the evaluation of these new materials.

We have also studied the possible harmful effects of increased fibrinolytic activity such as hemorrhage, proteolysis of clotting and other proteins, effect on vital organs, antibody formation and sensitization.

During our clinical trials it became obvious that certain patients had a marked resistance to fibrinolytic agents, and that these were frequently patients with chronic disease such as cancer and chronic infections or postoperative patients. Much of our investigation on animals has been designed to elucidate these problems.

RESULTS

In the course of these investigations from 1957 through February 1960 we have treated 197 patients with approximately 700 infusions of fibrinolysin and/or streptokinase. This does not include our original patients treated with makeshift materials and methods prior to 1957.

It includes all patients treated for thrombotic or embolic disease (Table 1).

TOXICITY

Toxic Reactions: In our original publication we reported a reaction rate of 48 per cent in 136 infusions. In the series of patients to be reported on we used only commercially available materials. A progressive improvement in the materials available for treatment was observed. Prior to 1958, using eleven lots of material from one company our reaction rate was 70 per cent for thirty-two patients (44 per cent serious) and 47 per cent (35 per cent serious) for the fifty-nine infusions given. Using twenty lots of fibrinolysin of another manufacturer prior to 1958 reactions were noted in 56 per cent of the patients and 48 per cent of the infusions In 1959, with an improvement in methods of preparation of streptokinase, there was marked improvement in the reaction rate with both fibrinolysin and streptokinase obtained from this same producer, with total reaction rates of approximately 27 per cent of patients and 17 per cent of infusions administered, and none of these were serious reactions. A rise in temperature of 1° F. when taken at hourly intervals was considered a reaction. Severe reactions including temperature rise of over 2° F., chills, pain, blood pressure changes, or other significant complications occurred in only 12 per cent of the patients or 6 per cent of infusions. With another streptokinase reported by Sherry as not producing any reactions we found the reaction rate to be 20 per cent. Perhaps the fact that we used no concomitant therapy such as cortisones or antihistaminics explains the difference.

Reactions other than chills and fever have been so infrequent as to be almost non-existent. Hypotension which occurred frequently prior to 1958 has practically never occurred since then. Abdominal pain and nausea and vomiting attributable to the fibrinolysin treatment have occurred in only four patients, and pain in bone or muscle has occurred in only one patient. These were seen always in patients given a second or third course of treatment, which was never carried out prior to 1959 or in patients with open, chronically infected wounds.

Hemorrhage and Changes in Blood Clotting Factors: Bleeding might be expected as a complication of active fibrinolysis. We have observed this only four times. Two instances occurred in patients who had recently undergone transur-

TABLE II

Methods of Evaluation of Results in Treatment of Venous Thrombosis

Degree of Improve- ment	Pain Gone (hr.)	Cuff Test Normal (hr.)	Measurements After Five Days
++++	24	24–36	Normal
+++	48	48	Minimal edema
++	48	72	Slight edema
+	More than 48	More than 72	Moderate edema
0	No change	No change	No change

ethral prostatectomy. A third who had melena was a patient who had a separation of the gastroenterostomy following gastrectomy for treatment of a peptic ulcer. The fourth was a patient with extensive metastatic carcinoma who bled from the gums with very active fibrinolysis. Only one of the patients (following prostatectomy) had severe bleeding.

Changes in prothrombin time and other clotting factors have been minimal. Only one patient with severe hepatic disease and significant prothrombin abnormality prior to treatment showed severe depression of her clotting factors and these returned to satisfactory levels rapidly with administration of vitamin K₁ oxide. Fibrinogen level is decreased in about 50 per cent of patients, but never to critical levels even with twelve to twenty-four hours' treatment and it quickly returns to pretreatment levels.

VENOUS THROMBOSIS

Although in experiments on animals it would appear that thrombi of more than seven days' duration and perhaps less are resistant to lysis, we undertook to treat all patients with venous thromboses regardless of the age of the thrombis. Ninety-eight patients were treated. Evaluation of results in patients with venous thrombosis is extremely difficult. We have used the cuff test, the ability to use the leg and leg measurements as our prime criteria for improvement (Table 11).

In a few patients venograms or postmortem examinations have objectively determined patency of the thrombosed vessels. In other patients the fact that blood could be withdrawn with ease from a vein which was previously completely filled with thrombus has been considered definitive proof of lysis. All patients were

TABLE III
Results of Fibrinolytic Therapy in Ninety-Eight Cases of
Venous Thrombosis

D	AT-	Im	Average		
Duration	No.	Good	Fair	None	Treatment (days)
Acute, 5 days 5 days to 2	48	40	7	1	2.8
weeks	17	13	1	3	3.5
More than 2 weeks	23	13	4	6	4.1
Tumor or me- chanical ob- struction	10	0	0	0	3.0

permitted to use the extremity the day after pain disappeared or as soon as softening was obvious, if pain was not a factor.

Forty-eight thromboses were of less than five days' duration (Table III). Here, as would be expected, the results were excellent, with thirty-six or 75 per cent showing complete regression and four showing marked improvement. Seven showed definite improvement and there was only one patient who fell into the class of minimal or no improvement. Days of treatment averaged 2.8.

There were seventeen patients who had had thrombosis for five days to two weeks. Here the results were still satisfactory with six showing complete recovery, seven showing marked improvement and one showing only moderate improvement. Three patients showed no improvement. Four of these patients required six days of treatment with an average of 3.5 days of treatment.

There were twenty-three patients whose thrombosis was of more than two weeks' duration, some up to several months. Here the results again showed a surprising number of patients (thirteen) with complete or marked regression. Four also showed moderate improvement. However, six or 26 per cent showed minimal or no change. Again there was an increase in the number of infusions used with four having six or more days of treatment and the average number of treatments being 4.1.

Ten patients undoubtedly had obstruction by tumor, but were treated because of the possibility of associated thrombosis. None of these improved significantly. This may be of significance in the consideration of the anti-inflammatory effect.

TABLE IV
Results of Treatment of Pulmonary Emboli

Result	No.
Excellent	8
Good	1
Death*	3

^{*} Of the three patients who died, emboli were demonstrated at postmortem examination in one.

PULMONARY EMBOLI

Twelve patients have been treated for pulmonary emboli (Table IV). Of these, one had an acute massive embolus and died of pulmonary edema within two hours of beginning treatment. Effective levels of lysis had not been reached. Ten patients had had multiple emboli over periods of two days to three weeks. In two, emboli continued to develop despite treatment with anticoagulants. Both had complete relief although one (with carcinoma of the pancreas) had a recurrent embolus and died one month later despite administration of anticoagulants. Seven of the remaining eight patients had complete relief of the symptoms and signs of pulmonary embolus. The seventh patient died of her disease, but at postmortem examination no thrombi were found and the cause of death was recorded as lymphangitic spread of cancer and pneumonitis.

The last patient was seen in shock while receiving norepinephrine, after three days of treatment for an acute embolus. She received four courses of treatment and no embolus was demonstrated at postmortem examination.

ARTERIAL THROMBOSIS AND EMBOLISM

With peripheral arterial thrombi and emboli, diagnosis and evaluation are less difficult than with venous thrombosis, pulmonary emboli or coronary and cerebral thrombosis. The results of treatment are therefore more clearcut, and subject to definitive evaluation. Spontaneous return of circulation following a definite obstruction for several hours is so rare as to be practically non-existent and return of circulation during the course of treatment would be a completely unexpected coincidence. The results of local treatment are even more definitive where the clot is demonstrated at operation and then disappears under direct vision.

The results of treatment since 1953 have

Table v

Results of Various Types of Therapy in Thirty-Six Cases of Arterial Thromboembolism

Time and Treatment	Total	Results		
	Total	Good	Improved	No Change
Prior to 1958 Since 1958 Systemic	6	0	0	6
only	17	5	4	8
Local only Fibrinoly- sin plus	7	4	0	3
femoral embo- lectomy	6	4	1	1

indicated the amount of systemic therapy necessary for effective response. Because of materials available this has been possible only in the last one and a half to two years. It is obvious from theoretic considerations and experiments on animals that a higher and more prolonged fibrinolytic activity will be necessary to treat arterial conditions.

Thirty-six patients with arterial occlusions have been included in this study (Table v). Six of these were treated prior to 1958 with totally inadequate dosage. None of these patients showed definite improvement although two showed some return of circulation during the course of treatment.

Systemic Fibrinolysin Therapy: Twenty-one patients were treated by systemic therapy only, seventeen since 1958. Of these, nine had thrombosis of a major vessel or vessels, four had emboli, two had thrombosis of a bypass graft and two had probable mechanical obstruction by tumor. Of these, six were seen within the first twentyfour hours after thrombosis or embolization. Five of these (80 per cent) had return of circulation with survival of the entire limb. The smallest amount of treatment associated with recovery was in a patient with cirrhosis of the liver and thrombosis of the femoral artery who received 800,000 units in two courses on the same day for a total of eight hours. One patient had a saddle embolus with thrombosis, one an embolus of the profunda femoris, one an embolus of the brachial artery, and the fifth had thrombosis of the ulnar artery. The one patient who failed to respond had associated heart failure; he died within twelve hours.

Of the six patients seen more than twentyfour hours after apparent occlusion, four showed improvement. Two patients died within twelve hours.

Of the six patients seen more than twenty-four hours after apparent occlusion, four showed improvement: two could ambulate without pain and without gangrene, one had the level of demarcation lowered from mid-calf to finally involve three toes only and one had return of circulation in one leg but not in the other. The other two had no obvious improvement. One with a thrombosis of several weeks' duration also had a cerebrovascular accident and died of cardiac decompensation. Only one had apparently adequate therapy without improvement.

Four patients remain of the group treated since 1958. One was a patient with severe trauma to the knee with a known thrombosis and also mechanical damage to the popliteal artery and infection. Two were patients in whom thrombosis in a bypass graft developed; both failed to show any response. The fourth was a patient who was thought to have a femoral embolus, but who had two operations, one in the femoral region and one in the iliac artery, without a clot being found. Therapy one day later failed to improve the leg.

Local Instillation of Fibrinolysin: It is with the local installation of fibrinolysin or operative embolectomy and fibrinolysin treatment that the most satisfactory results are obtained.

Seven patients were treated by local instillation of fibrinolysin into the occluded artery (Table v). Four of these were treated personally by direct instillation through a needle and polyethylene catheter into the thrombosed segment within sixteen hours to three days after the thrombosis. Dosage varied from 75,000 units in thirty minutes to 100,000 units in fifteen minutes. All patients showed complete return of circulation with almost complete lysis of the thrombi. Two were carotid artery thrombi of two and three days' duration, one an iliac through femoral to popliteal thrombus of sixteen hours' duration and the fourth a superior mesenteric thrombus of twenty minutes' duration following ligation at the time of removal of a Wilm's tumor in a child. Another patient had lysis of a carotid thrombus secondary to ligation for a subarachnoid hemorrhage, with return of function. Unfortunately, as might be expected, hemorrhage recurred from the aneurysm. Two patients had completely

inadequate dosage by percutaneous injection of a femoral artery. Both patients had gangrene and fibrous and calcific occlusion of the vessels rather than thrombosis. One patient treated in another hospital by irrigation rather than instillation failed to respond.

Fibrinolysin Therapy Plus Embolectomy: The six patients with systemic or local instillation of fibrinolysin plus embolectomy represent such varied types of treatment that no categorization is possible. Of the six patients treated by these combinations, four had a complete return to normal; there were two failures. One of these so-called failures did well after the embolectomy and local fibrinolysin but three days later had a massive hemorrhage into the calf secondary to anticoagulant therapy. The other was a patient with unsatisfactory timing who improved after fibrinolysin treatment but was operated on too late. It can be said in general that in the patient given fibrinolysin in adequate dosage, the embolus will be free from propagating thrombus if operation is carried out within one to two hours and it will not be attached to the arterial wall. Several patients who had poor backflow after thrombarterectomy or embolectomy had excellent flow after the instillation of fibrinolysin.

VESSELS OF THE EYE

To evaluate the effect of fibrinolytic activity on thrombi one might expect the retina to be ideal since the vessels can be directly visualized. Unfortunately, the results in eight patients are difficult to evaluate due to differences of opinion among the attending ophthalmologists.

One patient with a complete arterial occlusion had complete lysis with normal filling of the artery after two days of treatment but vision did not return. One patient was sent to New York Hospital from another institution with a diagnosis of thrombosis of the retinal artery. My impression was that filling of vessels had occurred during and after treatment. The third patient with thrombosis of the retinal artery was followed up by an ophthalmologist who believed that improvement was greater than he had anticipated. One patient received completely inadequate treatment without any change. A fifth patient had no significant improvement.

Three patients with thrombosis of the retinal vein showed rapid clearing of the vitreous and although agreement could not be reached as to lysis of thrombi or improvement of vision, none showed further progression although they had shown progressive changes over a period of five days to more than three weeks prior to treatment.

CORONARY OCCLUSION

We have treated only five patients with coronary occlusion. The results of a larger cooperative study are reported in this Symposium.

Our first patient was treated in January 1957. She was seen originally for treatment of an arterial thrombosis, and while being evaluated had severe pain in the chest and cardiac arrest. This was treated successfully but blood pressure remained at 76/40 mm. Hg. With an adequate dose of fibrinolysin the blood pressure rose to 100/60 mm. Hg and she responded normally. The arterial circulation returned. The patient remained well for sixteen hours when she suddenly had another attack and died. Postmortem examination showed an acute coronary thrombus and multiple fresh and old pulmonary emboli.

The second was a patient with known severe angina in whom a fourth proved coronary occlusion developed. He had very severe pain unrelieved by administration of nitroglycerin, 50 mg. of meperidine administered intravenously followed by administration of 150 mg. of morphine repeated at two- to threehour intervals. He was started on fibrinolysin at 2:30 P.M. By 6 P.M. he was free of pain and required no narcotic. At 2:30 A.M., after twelve hours, the infusion was stopped and he did well until 5 A.M. when pain recurred. We were not called and at 12 noon administration of anticoagulants was started; the patient again required large doses of narcotics. He died at 6:30 A.M. the next day. The postmortem examination showed pipe-stem coronary arteries with a thrombus present in the circumflex branch of the left coronary artery but with evidence of recanalization and a subendocardial infarct.

Two patients have been treated more recently with survival but without significant change in their course. Both were receiving vasopressor agents prior to treatment.

The most recent of our five patients was treated at another hospital this year. He had severe angina for several years with two previous coronary occlusions and a severe recurrent occlusion with anterolateral and posterior infarcts. He had a six-hour course of treatment on January 13, 1960, with relief of pain, relief of cyanosis and decompensation and elevation

of blood pressure. At 1 P.M. on January 14, 1960, he had recurrence of crushing pain in the chest and a fall in blood pressure. The pain was partially controlled with administration of Dilaudid. At 8 P.M. fibrinolysin administration was started again and was continued for eight hours. He had marked improvement in two hours and with other usual cardiac treatment became so well and free of pain that further therapy was considered unnecessary. The patient has remained much improved over his preinfarct state for over one month.

CEREBRAL THROMBOSES

We have treated ten patients with the clinical diagnosis of cerebral thrombosis or embolus. They are reported on in detail in this Symposium. For the sake of completeness it should be stated that two of these patients, one with thrombosis of three days' duration, the other with emboli of about eight hours' duration, showed rapid and almost complete return to normal. Two patients showed marked, unexpected improvement, one progressed as well as could be expected and only three showed no obvious improvement.

PRIAPISM

Priapism due to thrombosis of the penile veins and corpus cavernosum has been resistant to all types of treatment including administration of anticoagulants. Four of these patients have been treated by local instillation with permanent detumescence within forty-eight to seventy-two hours. Systemic infusion in one instance resulted in temporary improvement but adequate treatment had not been given.

MISCELLANEOUS CONDITIONS

Twenty patients with miscellaneous conditions of an investigational nature are included in this series. This includes two patients thought to have thrombosis of the renal artery. Neither was affected significantly. One was shown to have renal infarcts at postmortem examination; the other acute nephritis. One patient with intracranial infection following an operation on his eye was thought to have cavernous sinus thrombosis. Unfortunately, an autopsy on the patient was not performed. One patient with a transverse myelitis of two weeks' duration failed to show significant regression. One patient with a supposed thrombosis of the labrynthine artery had no improve-

TABLE VI

Results of Treatment in Four Patients with Experimentally Induced Venous Thrombosis

Results	No.
Total	4
Good	2
Temporary	1
Partial	1

ment with minimal treatment. Two patients with chronic lymphedema of three years' duration and five years' duration, respectively, showed marked improvement after four and six adequate courses of treatment, respectively.

Eleven patients were studied for the effect of fibrinolytic activity on cellulitis of the leg with edema. Only one course of treatment was used. No anti-inflammatory effect was observed. It should be noted, however, that patients with abscesses treated adequately in the course of thrombolysis showed rapid liquefaction and cure of the abscesses. At least two of these abscesses were due to resistant staphylococcus infections. Two patients with chronic bronchitis and/or bronchopneumonia responded by increasing freedom of expectoration and a rapid defervescence of fever.

INDUCED THROMBI

Four patients with chronic thrombophlebitis, lymphedema and static ulcers had thrombi produced in the vein of the arm by administration of sodium morrhuate (Table vi). One was treated with Lederle streptokinase (SK) and three with three different concentrations of SK per milligram of plasminogen. Two thrombi lysed completely and remained lysed. One lysed but recurred in twenty-four hours and the fourth did not lyse completely. No direct correlation with dosage could be determined.

COMMENTS

In evaluating the effect of treatment by fibrinolytic agents it is first necessary to determine the activity induced in vivo in the patient.

Applications of Experimental Results to Clinical Therapy: In experiments on animals it has been shown that fibrinolysin (streptokinase-activated plasminogen) will result in lysis of thrombi produced in veins and arteries, including the coronary arteries, if an adequate fibrinolytic activity is maintained (<1 hour lysis

time) for two to six hours depending on the site. Using undisturbed vessels, a higher fibrinolytic activity maintained for a longer period was necessary for arterial than for venous thrombi. In contrast, when the vessels were stripped as in the Wessler technic, thrombi in the two situations responded in essentially the same manner. This would indicate that the difference is primarily due to a mechanical factor. Contact with the thrombus at several points would obviously be a distinct advantage.

In essence a thrombosed artery is an end vessel; therefore activator or enzyme must be diffused through a static column of blood from one end only, reaching the clot with diminished concentration. With venous thrombi, the enzyme strikes the clot from both ends and through many collaterals at the same time.

In transferring results in animals to human beings, the same relative dosage was first used, approximately 1,000 units/kg./hr. for three to four hours. However, in general, even with venous thromboses, results were unsatisfactory. In many patients with this dosage it was impossible to obtain even good fibrinolytic activity in the plasma by in vitro tests. It then became apparent that inhibitors were present in most of our patients. Subsequently it has been shown that significant levels of inhibitor are present in most patients with chronic diseases and in postoperative patients after one week. They are less likely to be present in persons with acute thromboses who were otherwise well. Unfortunately these patients are in the minority.

Some of our patients, particularly those studied at Bellevue Hospital, have much lower inhibitor levels and a few have moderate spontaneous fibrinolytic activity. It is obvious that these people would require much smaller amounts of fibrinolysin. These facts may explain the good results reported by some workers with smaller amounts of material than we used.

In general, results of treatment can be correlated with the degree and duration of fibrinolytic activity obtained although this is not 100 per cent correct. Even with good activity some patients fail to respond; this was at first difficult to explain. However, the *in vitro* and animal experiments which indicate that increased fibrinogen levels make the clot more resistant to lysis are probably an important factor since many of these chronically ill patients have relatively very high fibrinogen levels.

When treatment of arterial lesions is attempted

even more serious factors of difference between animals and patients make their appearance. In animals we were dealing with thrombi formed in normal vessels. Almost all patients have some degree of atherosclerosis and most have marked narrowing and tortuosity. This increases the mechanical difficulties of diffusion of enzyme to the clot. Other unknown factors which may be of importance are the effect of atherosclerotic material itself on clotting and as an inhibitor of fibrinolysis. The fact that arterial thrombi are frequently found at the site of rupture of atherosclerotic plaques may be of significance. The effect of the normal endothelium in furthering fibrinolytic activity is suggested by Kwann and McFadzean.25 The other obvious mechanical factor of contractibility and mobility of an uninvolved vessel as compared with an atherosclerotic one must also be evaluated.

It became obvious early that infusion times of ten to twelve hours or more would be necessary for treatment of most arterial lesions, and increased concentrations would probably be very desirable. The effect of therapy must be more rapid with arterial occlusions since irreversible damage of tissue supervenes rapidly even in extremities. With the retina, brain and myocardium and even with massive pulmonary emboli even more rapid therapeutic response must be obtained early in the course of the disease. Increased purification was now essential, and became our prime purpose. It it important to remember that to this date pyrogenicity of streptokinase can be tested only in human beings since animals do not respond in the same way. It is hoped that some other test will be developed since the dangers in clinical trials are apparent.

Streptokinase versus Fibrinolysin: There has been much argument about the relative merits of streptokinase alone and fibrinolysin (SK and plasminogen). It seemed to us that this could not be settled on theoretic grounds or in vitro tests alone and it certainly cannot be established in animals. For proper evaluation it was essential that the fibrinolysin to be used be activated with the same or practically identical streptokinase as that used for streptokinase treatment alone. Using such materials we have found that fibrinolytic activity and therapeutic responses are quite similar. With less purified materials reactions are more severe with streptokinase alone as was shown in 1957. However, with more purified streptokinase this

may not hold true. Hemorrhagic phenomena are perhaps more frequent with streptokinase alone than with fibrinolysin. It should be noted that Fletcher et al. 18 reported hemorrhagic phenomena as one of the complications of prolonged streptokinase treatment; they used cortisone to control this hemorrhagic tendency.

Reactions to Therapy: It is important to note that in our own experience we have not used any drugs to prevent or control reactions except in a few patients (less than ten) in whom allergic histories were serious or who had had one serious reaction and whom we thought it urgent to treat again. We have rarely used anticoagulants with or even following fibrinolytic therapy and despite this we have had only two recurrences of thrombosis in the course of immediate follow up. Both of these occurred in patients with malignancy, one of whom had failed to respond to more than adequate anticoagulant therapy prior to excellent response to fibrinoly-sin therapy.

Other dangers of fibrinolysin treatment which have been suggested are: (1) pulmonary embolization with venous thromboses or peripheral embolization with central arterial thromboses. No patient under treatment in this series has had a pulmonary embolus. Indeed, the patients with thrombophlebitis and pulmonary emboli before treatment have responded by relief of the pulmonary embolic phenomena and have had no further evidence of embolization except in the patient previously mentioned, who was resistant to anticoagulation and had recurrent thrombophlebitis; (2) hemorrhage; (3) reactions to protein-split products of plasmin proteolytic activity. From the data available it would seem that this is not a factor to be seriously considered, at least with the doses used at present. There is no correlation between reactions and the amount of activity, either activator or proteolytic, obtained; and (4) sensitization. Streptokinase is an obvious antigen and would be expected to produce significant reactions if reused. The only severe reactions we have observed in the last year have occurred with patients who have had retreatment with streptokinase after periods of two to eight weeks. This in our experience is definitely worse with streptokinase alone, although it does occur with fibrinolysin activated with streptokinase. Of course the question arises: Is the reaction to fibrinolysin due to free streptokinase present in the mixture? Despite these enhanced reactions we have not hesitated to

repeat treatment when necessary but usually adding antihistaminics and rarely corticosteroids.

After eight to twelve weeks' treatment with fibrinolysin we have not seen severe reactions with repeated infusions. However, at any time after treatment with streptokinase alone there is the obvious effect of increased antistreptokinase levels.

Dosage Schedules: It should not be considered that these treatment methods, dosage schedules or methods of activation are the best or only means of obtaining results in the treatment of thromboembolic conditions. This presentation is intended only as an outline of our methods and reasons for arriving at our treatment schedules. It is likely that in acute venous thromboses we have overtreated some of our patients.

The original reason for the divided dose technic was to limit pyrogenic and other toxic effects. We have continued this technic when better materials were available because of convenience to us, the patient and the nursing staff. More recently other theoretical reasons have prompted us to continue. We have noted that succeeding daily infusions seem to give somewhat increased activity. Also, it is conceivable that old thrombi are altered by the first infusion so as to be more susceptible to subsequent lytic activity. In animals single infusions will certainly not lyse old clots. Animal experiments with divided dosage must be conducted and the effect of divided as compared with continuous infusion must be evaluated more thoroughly in human patients.

We have rarely seen significant clinical response in a patient who did not show at least moderate fibrinolytic (four hours lysis time) activity, if adequate samples were obtained. In the past year, for reasons of convenience and also to prove that with these doses treatment could be managed without the detailed laboratory studies, we have treated many patients with only minimal laboratory control available in any hospital and even without any control in several patients. In conjunction with Richter and his group we have treated a large series of patients (over sixty) with only prothrombin time and whole clot lysis determinations, even in those with coronary occlusion and cerebral thrombosis. The chief difficulty here is that we cannot be certain that in patients who gave poor results there was enough material given to obtain activity.

Although we have used relatively large

amounts of fibrinolytic agents it is not certain that they are always necessary. Some patients are more sensitive than others. However, we believe that the minimum amounts suggested for one product (Actase®) would not be sufficient for effective results in our patients. We have had experience with other fibrinolytic materials including urokinase-activated and spontaneously-activated human fibrinolysin and bovine fibrinolysin, and have failed to observe significant effect. This does not mean that with different preparations or methods of treatment they would not be effective.

Enhancement of Fibrinolytic Activity: We have been particularly interested in methods of enhancing the effect of these materials in patients. The simplest effective method is fasting or at least the use of a diet low in animal fat. Certainly, heavy meals or meals particularly high in fat content inhibit the fibrinolytic activity. Unsaturated vegetable fats such as safflower oil are not inhibitory and may actually enhance activity. Exercise, if possible, will enhance activity. Attempts to decrease inhibitor activity prior to infusion of fibrinolysin have not been successful as yet but our experience is limited and we believe this is an investigation that should be extended.

We have not had any experience with pyrogens. However, with our pyrogenic responses to streptokinase and fibrinolysin we have not noted any marked increase in fibrinolytic activity. Indeed, sometimes it has been less than expected with the fibrinolytic material used.

SUMMARY

The general results of treatment of 197 patients have shown that effective fibrinolytic activity can be delivered and maintained by either fibrinolysin (SK-activated) plasminogen or streptokinase alone for periods of up to twenty-four hours. In general, much shorter periods of treatment (four to six hours with doses of 200,000 to 600,000 units) repeated for two to four days are necessary for treatment of venous thromboses, depending on the age of the condition and the amount of inhibitor present. Arterial thrombi or emboli are best treated by local instillation followed by removal of the embolus if present, or by local plus systemic treatment. Thromboses can be treated by more prolonged infusions, usually of ten to twelve or more hours' duration. Coronary and cerebral thromboses must be approached with caution primarily because of the difficulty of diagnosis and evaluation. With carotid arteriograms and local infusion, treatment of early cerebral thrombosis can be undertaken more logically.

The materials available have steadily improved and are now safe for general use in the dosages recommended here and with proper laboratory control of activity and side effects.

Search should continue for better activators and for methods of enhancing fibrinolytic activity in the patient.

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DISCUSSION OF PAPER BY DR. CLIFFTON

DR. JAMES EVANS (Boston, Massachusetts): We have been especially interested in the venous area of the arm of patients who have undergone radical mastectomy and in whom a swollen arm so often develops. We believe that this is the result of thrombophlebitis of the brachial, axillary and subclavian veins. All these patients were treated with Actase in the past when violent reactions were noted. For example, in one patient there was a thrombus where the axillary vein joins the brachial. With treatment the channel opened. However, a stricture was still present, but it was at the point of ligation of the subscapular vein at operation. All swelling disappeared and the patient has remained well as of the last examination, one year after operation. This thrombus was nine days old, but it is difficult to be sure of the age of a thrombus because clinical evidence is the only indication of the onset of tenderness. Moreover, many of these patients have tenderness of the arm and pain in the axillary area. In another patient who received only 25,000 units of Actase almost complete reopening of the axillary vein seems to have occurred. She had a violent reaction to Actase and her husband refused permission for another injection to be given. The stress and charge of adrenalin helped contribute to the good effect, in spite of the fact that she had received only 25,000 units. In another patient who received 50,000 units in one day there was dramatic reopening of all four veins. Here again, we wonder how much help is derived from the pyrogenic effect in bringing about a good clinical cure. Another patient had reopening of the brachial vein to the subclavian vein and the restoration of valves.

DR. W. P. CHAPMAN (Boston, Massachusetts): Dr. Cliffton, what are your criteria for the various grades of improvement in the patients with pulmonary embolism who were treated.

DR. GEORGE KLEINFELD (New York, New York): Dr. David Habif and I have treated nineteen patients with intravenous fibrinolysin who had acute or chronic lymphedema of the arm following radical mastectomy. The rationale was the possible partial blockage of the lymphatic absorptive bed and trunks by fibrin secondary to an acute or chronic infection. In several patients there occurred marked softening within one to three days but improvement was not maintained. Two patients treated shortly after the onset of an attack of acute cellulitis and edema were considered to have improved more rapidly than a comparable series receiving antibiotics alone. The over-all results were equivocal. While we agree that an occasional patient has the additional factor of venous obstruction augmenting the lymphedema, the patients in our group definitely had normal axillary and cephalic veins.

Three patients in this group were treated with intravenous administration of streptokinase. Of these, two patients were given initial injections of 100,000 units with sustaining injections of 25,000 units per hour for four hours. In one of these patients who had bumped her thigh shortly before the infusion, a hematoma about 15 cm. wide developed in the bruised area halfway through the infusion. She also began to bleed from a dentureirradiated area on the gum. The other patient, who had a granulating wound on the wall of the chest, began to ooze blood from the wound and from venapuncture sites which had been made before the infusion. We consider these incidents to be minor additional evidence supporting the fact that streptokinase-induced fibrinolytic activity can produce lysis of clots in vivo, but certainly these effects make

us approach the treatment of postoperative patients with great trepidation.

FROM THE FLOOR: Dr. Kleinfeld, were these patients receiving anticoagulants?

Dr. Kleinfeld: No, they were not.

Dr. J. L. Ambrus (Buffalo, New York): Dr. Cliffton discussed briefly the relative values of streptokinase vs. streptokinase-activated plasmin therapy. We believe that the chief advantage of streptokinase-plasmin over strepotokinase alone is that the former therapy is not severely inhibited by pre-existing antistreptokinase antibodies and that one can obtain therapeutic results with less total streptokinase infused into the patient. Therefore, one would expect less streptokinase antibody production following therapy,

with less danger to possible retreatment.

DR. EUGENE E. CLIFFTON (New York, New York): Most of the patients with pulmonary embolism had multiple emboli and had severe symptoms at the time we treated them. All had complete relief of their symptoms during the course of their first treatment and did not have any additional pulmonary emboli subsequently, in spite of the fact that administration of anticoagulants was discontinued. The treatment schedule we use entails a much larger dosage than most others have indicated. We use at least 50,000 units per hour and sometimes use as much as 100,000 units per hour for four to six hours. In a patient with an acute pulmonary embolus we have tried as much as 200,000 to 300,000 in the first hour which I think essential for such a case. This may also apply to the patient with coronary thrombosis in the acute stage. We are now willing to use 200,000 or 300,000 units in the first hour. In cerebral thrombosis the mode of treatment will be local injection after the carotid angiogram is obtained. Using the material locally, it is possible to cut the dose markedly. With arterial thrombosis we must use 100,000 units per hour for ten to twelve hours to obtain systemic effect in the thrombus. There are many reasons for this, such as (1) atherosclerosis with narrowing in patients as opposed to animals, and (2) the local effects of atherosclerotic plaques as inhibitors. However, with local use in these same situations, you can inject 50,000 to 100,000 units locally in ten or fifteen minutes and lyse the clot completely. I am convinced that local use is the better way. A few patients with emboli have been treated systemically while being prepared for surgery. In these we found that the emboli just dropped out of the artery. There was no propagating thrombus and the embolus was not stuck to the wall. You do not have to ream them out as must be done ordinarily.

Streptokinase as a Thrombolytic Agent*

ALAN J. JOHNSON, M.D. and W. Ross McCarty, M.D.

New York, New York

EXTENSIVE clinical studies in many centers have indicated the thrombolytic potential of various intravenously infused plasmin preparations. Interpretations of these clinical studies are complicated by (1) wide variations in the natural course of disease; (2) an anti-inflammatory effect which may follow clinical use of the enzymes; and (3) inadequate definition and standardization of these plasmin preparations containing varying amounts of streptokinase, streptokinase-activated plasminogen as the activator complex, and streptokinase-activated plasminogen as plasmin.

In the present studies, forty-one experimental thrombi, 2 to 5 inches long, were induced in the superficial veins in the arms and legs of human volunteers by direct irritation of the intima with a dental broach or by chemical irritation with sodium morrhuate. The position and size of the thrombi were determined by clinical observation and by venograms. Various fibrinolytic systems were then produced by infusing streptokinase (SK) into a contralateral extremity at varying time intervals after formation of clots. Similar studies were also made on a patient with a fibrinolytic system due to circulating endogenous activator. Thus, objective comparison was made of the various methods to produce thrombolysis and to prevent reformation of the thrombi.

Three different systems were established by varying the amount of SK infused: (1) moderate amounts of circulating plasmin with no measurable free SK (method P); (2) negligible plasmin with moderate amounts of free SK or activator (method SK); and (3) small amounts of both free SK and plasmin (method SK-P). Thrombolysis was also tested in a fourth system, moderate amounts of endogenous activator with negligible amounts of plasmin (method A).

These systems were produced and utilized under rigidly controlled biochemical conditions,

each system representing a different, sharply defined agent for the production of thrombolysis. Since circulating SK in human plasma could not be distinguished by assay from the activator complex of SK-plasminogen under these experimental conditions, the use of endogenous human activator also aided in the evaluation of the SK-activator complex as a thrombolytic agent.

Figure 1 represents most of the important components of the human fibrinolytic system. The kinases from tissue, plasma or streptokinase appear to react stoichiometrically with a proenzyme in plasminogen to form an activator complex. This complex, designated "activator," or the naturally occurring activator in urine, plasma or tissues, reacts catalytically with plasminogen (the proteolytic precursor) to form a proteolytic enzyme, plasmin. The plasmin in turn acts upon fibrin, fibrinogen and other proteins to produce soluble split products of these native proteins.

In order to produce pathologic fibrinolysis in man, this proteolytic system must be uninhibited. Normally, there may be interference with the system at three levels. Streptokinase may be neutralized by a specific antibody and by less specific inhibitors. Activator may be neutralized by inhibitors found primarily in the alpha-2 globulin fraction of

Fig. 1. Mechanism of fibrinolysis in man.

^{*} From New York University-Bellevue Medical Center, New York, New York. This study was supported by research grants from the U.S. Public Health Service, National Heart Institute, H-5003, Lederle Laboratory Division, American Cyanamid Company, Pearl River, N. Y., and the Life Insurance Medical Research Fund, G-59-23.

TABLE I

Streptokinase Antibody and SK-Plasmin Inhibitor in the Circulating Blood of Fifteen Patients Prior to the Intravenous Infusion of SK

Quantity	Antibody* (units)	Inhibitor (units)	Total Inhibition (Antibody and Inhibitor) (units)
Mean	195,000	175,000	355,000
Range	40,000 to 470,000	43,000 to 410,000	83,000 to 684,000

^{*} Proportion of antibody to total amount of inhibition in patients varied from 28 to 87 per cent.

the serum, and plasmin may be neutralized by another inhibitor found primarily in the alpha-1 globulin fraction.⁷⁻¹⁰

METHODS

A variety of assay procedures was employed. In general, the samples obtained for assay were refrigerated immediately after they were taken.

The fibrinolytic assays for sterptokinase, SK-activated plasmin, SK-activator complex and/or free SK, SK antibody, SK-plasmin inhibitor, plasminogen, fibrinogen and prothrombin have been described previously.⁶ The fibrinolytic equivalent of urokinase was used in place of streptokinase for the determination of activator-inhibitor.

The effect of plasmin on the whole blood clot lysis time was determined by adding 4 × 10⁻⁴ M of epsilon-aminocaproic acid to the lysis-time tube prior to clotting to inhibit the activator.¹¹

The euglobulin clot lysis time was performed by the method of Von Kaulla.¹²

Plasmin inhibitor was determined as previously described. 13

The method of Astrup and Müllertz¹⁴ was used for estimating the *fibrinolytic activity* by the fibrin plate technic.

Experimental thrombi were induced in man by previously described methods.⁶

RESULTS

In order to produce a fibrinolytic system by any one of the three systems previously enumerated, it was found to be necessary to give an initial or priming dose to just neutralize the circulating SK-antibody and alpha-2 inhibitors in each patient. Therefore, additional infused SK was free to produce active proteolysis. 6

The mean antibody, in a representative group of patients in this study, was 195,000 units and the range was 40,000 to 470,000 (Table 1).

The mean inhibitor, on the other hand, was 175,000 units and the range was 43,000 to 410,000.

It is evident that the range of each of these was very wide. Since the proportion of antibody to the total amount of inhibition also varied widely, from 28 to 87 per cent, it was clear that prior determination of the total inhibition was essential in the estimation of an appropriate priming or neutralizing dose.

EFFECTS OF STREPTOKINASE ADMINISTRATION

Priming Dosage of SK: The effect of such a priming dose on the plasminogen level of the plasma may be seen in Figure 2. The amount of SK given in the priming dose was expressed as a per cent of the calculated amount required to satisfy the total amount of circulating antibody and inhibitor in the patient's serum. In short, when the amount of SK, in per cent, was plotted against the logarithm of the plasminogen concentration in the plasma it was evident that the infusion of an amount of SK equal to the average amount of antibody present in the plasma produced little or no decrease in the circulating plasminogen. It was also apparent that the infusion of an amount of SK equal to or just greater than that represented by the combined antibody and inhibitor (100 per cent on the abscissa) produced a minimal change in the circulating plasminogen. It was concluded from these data and similar data on prothrombin levels that little or no free SKplasmin was formed until neutralization was relatively complete. The fibrinogen, complement and plasmin levels of the patient's plasma also supported this conclusion.

After neutralization of the antibody and inhibitor had been effected, additional SK was given in an amount appropriate for the production of one of the proteolytic systems

previously enumerated.

Small Dosage of SK: When small amounts of SK were given at this time (about 5,000 units per hour) moderate or large amounts of SK-plasmin were produced as in method P. As a result, the one-stage prothrombin time was markedly elevated and the fibrinogen was moderately or markedly depleted in this particular method with potentially serious effects to the patient. This system could be maintained for long periods of time by intermittent injections of only 10,000 to 20,000 units of SK at the rate of approximately 5,000 units per hour.

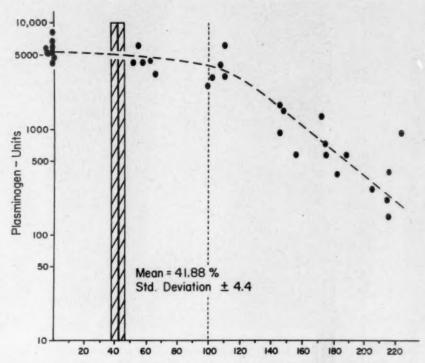


Fig. 2. Effect of intravenous infusion of priming and first sustaining dose of streptokinase on plasminogen levels of patients treated according to method SK-P (semilog plot). Dashed line represents a mean of values. At cross-hatched line, 41.88 per cent, amount of SK infused is equal to mean of total antibody in patient's serum; at dotted line, 100 per cent, amount of SK infused is equal to total antibody plus inhibitor in patient's serum.

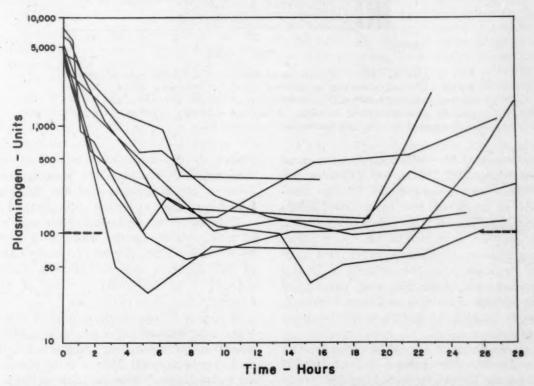


Fig. 3. Effect of intravenous infusion of streptokinase on plasminogen levels of patients treated according to method SK-P. For emphasis, an abscissa was drawn through 100 units of plasminogen on the ordinate.

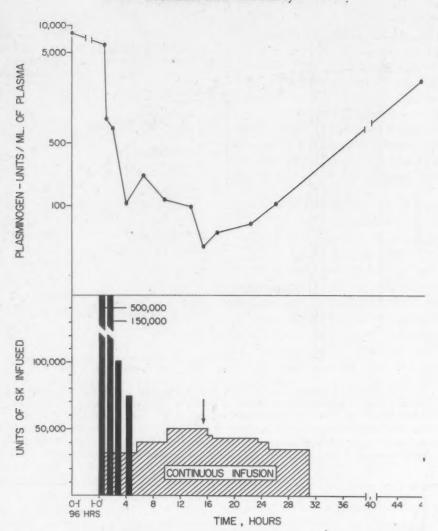


Fig. 4. Case 1. Effect of intravenous infusion of 2,145,000 units of streptokinase. Treated according to method SK-P for thirty-one hours. Ninety-six hours elapsed between the time preinjection samples were taken and the beginning of streptokinase infusion. Solid bars represent rapid injection of a major part of priming and first sustaining doses of SK.

Larger Dosage of SK: When large amounts of SK were given (200,000 to 400,000 units per hour) there was a very rapid fall in plasminogen to levels of less than 100 fibrinolytic units, and free SK was evident in the blood, without plasmin, as in method SK. In general, the coagulation constituents were not profoundly depleted with this system, if it was rapidly induced. Free SK was maintained for long periods of time, in vivo, by a sustained, continuous infusion of 45,000 to 60,000 units per hour.

Intermediate Dosage of SK: When intermediate amounts of SK were infused (about 100,000 units per hour for two to three hours) the plasminogen decreased rapidly to levels of 200 to 500 units, the prothrombin time rarely ex-

ceeded twenty-three to twenty-four seconds, there was little danger to the patient, and small amounts of SK-plasmin and free SK were released into the circulation as in method SK-P.

This system was maintained for long periods of time, in vivo, by the infusion of 25,000 to 45,000 units of SK per hour. Larger amounts of SK depleted the plasminogen excessively (below 75 to 100 units per ml. of plasma) causing reformation of the clot.

In Figure 3 the plasminogen levels in patients treated by method SK-P are shown during the period of infusion. For emphasis, an abscissa was drawn through 100 units of plasminogen on the ordinate. The duration of the infusion (about twenty-five hours) and the amount infused (plus or minus 3,000 units per hour) were

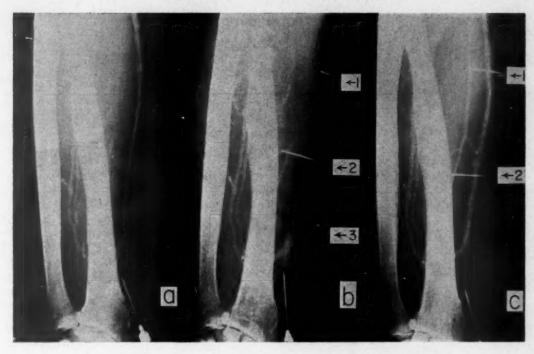


Fig. 5. Case 2. Roentgenographic demonstration of clot lysis; (a) prior to induction of the clot; (b) immediately prior to the SK infusion; 20 cm. clot induced forty-eight hours previously; (c) eighteen hours after SK infusion demonstrating that persistent clot lysis has occurred. Radiopaque lines (1) and (2) define original area of clot induction; arrow at (3) indicates detail portion of clot.

determined by following the various biochemical constitutents of the fibrinolytic system in the patient's blood, and by clinical and roentgenographic appraisal of the experimentally induced clot. It is important to note that the exact amount of SK required varied for each patient, and varied with time in each patient.

After clot lysis had occurred, reformation of the clot was prevented, in those patients with sufficient residual plasminogen, by prolonging the SK infusion for an additional four to six hours. The infusion rate was usually decreased, at this time, to provide an excess of SK-plasmin and plasminogen was evidenced in Figure 3.

ILLUSTRATIVE CASES

CASE 1. This sequence of biochemical events may be seen in a patient treated with 2,145,000 units of SK according to method SK-P, for thirty-one hours (Fig. 4). Two thrombi were induced in this patient, one in the left arm forty-eight hours prior to the infusion of SK, and one in the right arm twenty-four hours prior to the infusion of SK. A priming dose of 500,000 units of SK was given over a twenty-five-minute period, and 300,000 units were given during the next three hours to drive the plasminogen down to about 100 units. Then a continuous infusion was started at 30,000 and changed to 40,000 units per hour. Finally, the patient was given 50,000 units

per hour in an arbitrary attempt to reverse the lytic process which seemed to be progressing well. Shortly before the sixteenth hour (note arrow on Figure 4), the plasminogen decreased to 50 units and the twenty-four hour clot reformed. The infusion rate was promptly decreased, and thrombolysis started again. This type of experiment was performed repeatedly. When the infusion rate was not decreased immediately, the clot resisted all further efforts to lyse it.

Clinical observations and venograms the day after infusion revealed that both clots had lysed.

Case 2. The course of another typical patient, treated for thirty-six hours with 1,805,000 units of SK according to method SK-P, which produced small amounts of plasmin and free SK, is shown in the venograms in Figure 5. An intravascular clot was induced in this patient's right arm forty-eight hours prior to the infusion of SK, and the infusion was continued for six additional hours after clot lysis to prevent clot reformation. The venograms show conclusively that persistent clot lysis occurred. Lysis of the clot was clinically evident after the infusion had been running for thirty hours.

CASE 3. It was our good fortune to observe this patient with pathologic spontaneous fibrinolysis for approximately six weeks. His whole blood clot lysis time *in vitro* was approximately four hours. During this time, extensive studies were made, and it was concluded that the patient had an apparently isolated defect with an excess of endogenous cir-

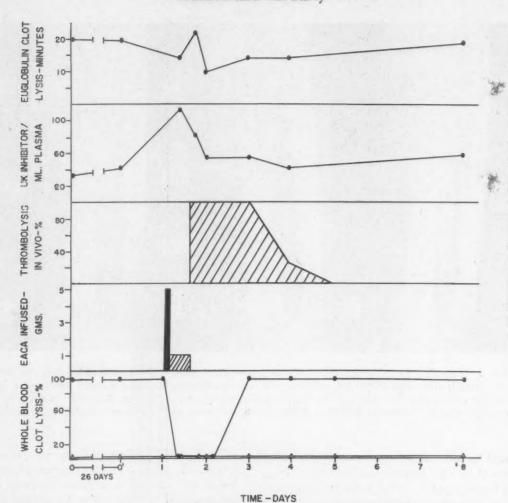


Fig. 6. Case 3. Experimental clot formation during the infusion of EACA and thrombolysis following the infusion of EACA in a forty-five year old man with pathologic spontaneous fibrinolysis due to endogenous activator. The euglobulin clot lysis, urokinase inhibitor and whole blood clot lysis with and without added EACA remained constant throughout the entire twenty-six days of the control period. \bullet = no EACA added in vitro. $\Delta = 4 \times 10^{-4}$ M EACA added in vitro.

culating activator. The evidence for this may be summarized as follows:

- A. Evidence for Endogenous Activator:
- (1) Lysis of unheated fibrin plate (bovine plasminogen added).
- Lysis of unheated fibrin plate (human plasminogen added).
- (3) Low activator inhibitor in plasma.
- (4) Rapid euglobulin clot lysis (20 minutes).
- (5) Decrease in plasminogen levels of plasma (1,200 units per ml.), the normal value being 5,000 units per ml.
- B. Evidence Against Endogenous Plasmin:
- (1) Absence of whole blood clot lysis (none in forty-eight hours) in vitro with 4 × 10⁻⁴ M of EACA.
- (2) Absence of marked decrease in fibrinogen level of plasma (180 mg. per cent).

- (3) Negligible lysis of heated fibrin plate (no bovine plasminogen).
- (4) Absence of low plasmin inhibitor in plasma.

It is noteworthy that little or no endogenous plasmin was produced and that endogenous activator from this patient's plasma was labile on incubation at 37.5°C. During the course of these observations, the patient was given infusions of the activator-inhibitor, EACA, 15.16 on several cocasions. Six hours after each infusion was started there was no measurable fibrinolytic activity in the whole blood or plasma (which did not lyse for over a week). This degree of inhibition continued for twelve to twenty-four hours following the infusion. The euglobulin lysis time, however, remained about twenty minutes.

During the course of three of these infusions, thrombi were induced in the superficial veins. After the infusions of EACA were discontinued, the fibrinolytic system returned and the thrombi began

TABLE II

Lysis of Intravascular Blood Clots in Man

Method	Nt6		No Lysis				
of Therapy	No. of Clots	Partial	Com- plete	Clot Reformed	Lysis Persisted	Total	Effected
P	7	3	1	4	0	4	3
SK	7	3	2	3 (1 to 48 hrs.)	2	5	(1 to 48 hrs.)
SK-P	11	0	. 11	0	11	11 (3 to 48 hrs.)	(2 to 48 hrs.)
A	3	0	3	0	3	3 (1 to 48 hrs.)	0
Готаl	28	6	17	7	16	23	5

to lyse. The course of one of these experiments may be seen in Figure 6. It may be noted that the thrombus was about forty-eight hours old before a strong fibrinolytic system occurred following the infusion of EACA. Thus, forty-eight hours elapsed before complete thrombolysis occurred. Two additional clots, twenty-four hours old, lysed within twenty-four to forty-eight hours after the EACA infusion was discontinued and fibrinolytic activity became evident.

COMPARISON OF METHODS

The relative effectiveness of the four methods are compared in Table II. It may be recalled that (1) moderate amounts of circulating plasmin were produced in method P with no measurable free SK; (2) negligible plasmin was produced in method SK with moderate amounts of free SK or activator; (3) small amounts of both free SK and plasmin were produced in method SK-P and (4) moderate amounts of endogenous activator were evident in method A with negligible amounts of plasmin.

Method SK-P: SK was infused in seven instances according to the method which produced SK-plasmin. Partial lysis was evident in three and complete lysis in one. Circulating plasmin was present in all without detectable free SK, but no persistent clot lysis occurred.

Method SK: SK was infused in seven instances according to the method which produced free SK. Two clots lysed and did not reform, but complete or incomplete lysis was followed by clot reformation in three. No lysis was detected in two. Thus, persistent lysis occurred in only two of the seven treated by this method.

With respect to the method used in the patient whose venograms are shown in Figure 5, that is, the production of small amounts of free SK and SK- plasmin, SK was infused in eleven instances. Persistent clot lysis occurred in all.

Method A: With regard to the patient with endogenous activator, three clots were induced in three separate instances, and persistent thrombolysis occurred in all three.

It is evident that persistent lysis did not occur with forty-eight hour clots unless method SK-P or method A was used. It was our impression that forty-eight hour clots took longer, and were more difficult to lyse than the twenty-four hour clots. Embolic complications were not observed with any of the methods.

Conclusions

From the foregoing, it is apparent that the optimum biochemical conditions to effect consistent and reproducible clot lysis in man and prevent clot reformation are created when SK is infused to produce small amounts of plasmin and free SK or activator in vivo.

Previous in vitro studies, primarily from Denmark, 17,18 and the United States, 18,20 and in vivo studies in animals from this and other laboratories 13,21,22 have demonstrated that fibrinolysis could be effected more readily by SK or activator than by plasmin alone. The present investigation has extended this observation to man, if the infusion of SK is carefully controlled to prevent excessive depletion of the patient's plasminogen.

The following hypotheses were formulated on the basis of these previous studies and the data presented herein:

1. Circulating plasminogen is adsorbed on the fibrin clot as it forms. Adsorption effectively removes the plasminogen from association with, and the effect of, circulating natural inhibitors of both plasmin and activator. When SK is utilized, it is infused in amounts calculated to neutralize the circulating antibody and inhibitors. Additional infused SK (or activator), circulating free in the blood stream, combines specifically with its substrate plasminogen which previously adsorbed on the clot. Thrombolysis occurs.

2. Intimal damage to the wall of the vein persists. Clot formation recurs, in varying degrees, adsorbing the available plasminogen, free SK and SK-plasmin (or activator) as it forms.

Thrombolysis recurs.

Method A, with small amounts of endogenous activator, and method SK-P, with small amounts of free SK, SK-activator complex and SK plasmin, proved to be equally thrombolytic presumably because of the adsorption of residual plasminogen on the reforming clot, together with endogenous activator, or free SK, SK-activator complex and SK-plasmin. In each instance, therefore, the clot which reformed adsorbed all the constituents essential for its own destruction.

The results demonstrate that SK or activator will consistently and reproducibly lyse intravascular clots and prevent reformation of the clots when used under precisely defined biochemical conditions.

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Discussion of Paper by Drs. Johnson and McCarty

DR. LOUISE LANG PHILLIPS (New York, New York): My discussion is based on some of the patients whom Dr. George Kleinfeld mentioned in relation to the previous paper. A careful study was made of the fibrinolytic enzyme in the blood of three patients who had been treated with streptokinase. All three patients were given 100,000 units of highly purified streptokinase in a priming dose and then an

additional 100,000 units were given as a slow infusion.

Case I is a patient who had been given Actase on two previous occasions and apparently had developed an immunologic resistance to streptokinase. The fibrinogen level remained quite steady, and the profibrinolysin level and the inhibitors did not change. A slight degree of plasma clot lysis was obtained during the infusion but this rapidly disappeared. This patient showed no ecchymosis or other evidence of bleeding.

Case 2, a patient with normal levels of all factors in the control sample, showed a decrease from 270 mg. per cent fibrinogen to 46 mg. per cent in the postinfusion sample and the profibrinolysin as well as the inhibitors decreased to almost nothing. Unfortunately in Case 3, a control sample was not obtained but a specimen of blood, drawn immediately after the priming dose, showed extremely low levels of profibrinolysin and inhibitors. The fibrinogen apparently had not yet been destroyed but by the end of the infusion the level had dropped from 330 to 130 mg. per cent and further decreases in the levels of profibrinolysin and inhibitors were evident. Rapid clot lysis occurred in both of these patients and the hemorrhagic conditions described by Dr. Kleinfeld appeared.

Subsequent studies at twenty-four, forty-eight and ninty-six hours show that forty-eight hours or longer were required before fibrinogen, profibrinolysin and inhibitor levels returned to normal. After the profibrinolysin level is depleted to this extent, it would seem fairly useless to continue infusion of strepto-kinase. Perhaps the administration of a fibrinolysin preparation instead of additional streptokinase would be more effective in preventing recurrence of thrombosis during this period.

DR. HORST ROSOLLECK (Buckenburg, Germany): We have studied the effect of streptokinase (Varidase®) on arterial thrombi in vitro. For this study we employed human arteries which had been occluded as a result of peripheral vascular diseases. The arteries were obtained from amputated limbs or by arteriectomy. The vessels were sectioned into lengths of 1 or 2 cm. and the sections placed in solutions of streptokinase varying in concentration from 500 to 10,000 units. To simulate physiologic conditions the experimental solution was maintained at a temperature of 37°c. Complete lysis of clots was observed in the six specimens which were tested. Control specimens exposed for the same length of time to streptokinase inactivated by heating showed no lysis. The ages of the clots tested were estimated at several months or older. In no instance were the media or adventitia of the arterial walls affected as confirmed by microscopic examinations. We believe that these observations support the theory of Sherry et al. about the mechanism of clot lysis by diffusion of the activator into the thrombus followed by activation of intrinsic plasminogen and by lysis of the clot.

Dr. Gabor Markus (Buffalo, New York): What Dr. Johnson really compares are low amounts of streptokinase and high amounts of streptokinase. He calls the low amounts of streptokinase, plasmin therapy and the high amounts, streptokinase therapy. I think it is clear that, if we want to have a valid comparison between two kinds of treatments, both have to be administered under optimal conditions. No proponent of the plasmin therapy would agree that the amount of plasmin that is generated by injecting low amounts of streptokinase constitutes plasmin therapy. I think that this point should be made clear and the two should not be confused.

DR. ALAN J. JOHNSON (New York, New York): It is evident that the first patient Dr. Phillips discussed had received large amounts of streptokinase antibody. This is an important problem that must be met by precise measurements and carefully regulated dosage requirements, or by the use of an activator preparation. In Dr. Phillips' second patient, the low plasma plasminogen was measured by the casein assay. An activator assay for plasminogen (which is about 100 times more sensitive than the casein assay) was used, and we reduced the plasminogen to less than one-tenth, and more than one-twentieth, of its preinfusion level. This is necessarily, then, an extremely precise determination for reasons that were previously discussed. At the time Dr. Phillips discontinued the infusion, therefore, we would continue infusing streptokinase, but in modera-

I wish to thank Dr. Rosolleck for his comments. We certainly agree with him.

I also wish to answer briefly two comments that were made previously. First, venograms may be made when patients have spasms in vessels by previously incubating a 1:4 dilution of 50 per cent Hypaque® in saline at 37.5°c. This was the only technic which permitted us to obtain venograms in those few instances where profound spasm had occurred. Secondly, we have not seen anaphylactic shock in any patients given streptokinase, regardless of the number and frequency of repeated infusions, although several thousand infusions of SK have been given over the past five years.

Finally, I wish to emphasize that our model was an experimental one to determine whether we could use the agents to lyse clots and keep vessels patent. We agree that anticoagulant therapy is highly desirable.

I think the efficacy of plasmin is still to be determined. In our own series, preliminary but not persistent lysis occurred in only one of seven clots when a high plasmin system was used. When a high streptokinase system was obtained, without any demonstrable plasmin, preliminary lysis occurred in four of seven clots. When a system was obtained, in vivo, containing streptokinase (or activator) and small amounts of plasmin, all eleven clots were lysed.

The Combined Use of Fibrinolytic and Anticoagulant Agents

Laboratory and Clinical Considerations*

KENNETH M. Moser, M.D. + and George C. Hajjar, M.D.

Washington, D. C.

ALL INVESTIGATORS studying the clinical application of fibrinolytic agents must carefully maintain the proper perspective in assessing the role of these drugs in the management of thromboembolic disorders in man. No degree of therapeutic enthusiasm should be permitted to obscure other important aspects of the problem which will not be solved even by the development of an "ideal" clot-dissolving agent.

The appearance of intravascular thrombosis should be considered a signal that some physiologic or structural aberration has disrupted the normal homeostatic mechanisms which assure fluidity of the blood. Viewed in this light, thrombosis properly becomes a symptom rather than a disease, a symptom which may be the common expression of diverse etiologic factors. Unless we accept such an orientation toward thromboembolism, our therapeutic efforts are likely to be less than optimal, for, without some attempt to control the factors which prompted the original thrombosis, this "symptom" may recur.

Since the days of Virchow, physicians have recognized that three fundamental abnormalities appear to be involved in thrombosis: (1) structural changes in the intima of vessels; (2) stasis of blood flow; and (3) disturbances in the coagulation mechanism. All therapeutic maneuvers previously developed to combat or prevent thromboembolism have been directed against one or more of these three fundamental "pathogenetic" factors. The advent of agents capable of dissolving fresh intravascular clots has not diminished the need for continued attention to this thrombogenic triad. Without

such attention successful clot dissolution may be promptly negated by clot reformation. Unfortunately, at present, we rarely can determine accurately the etiologic factors which have contributed to a specific thrombotic event; nor can we predict how long the responsible pathogenetic mechanisms may persist after therapy has been initiated.

Clot dissolution cannot be the only goal in treating thromboembolism any more than reduction of fever can be the goal in bacterial infection. The maintenance of vascular patency after clot lysis has been achieved is an equally important objective. Unless this is recognized, investigations in animals and in man designed to assess the efficacy of fibrinolytic agents will be subject to error.

METHODS FOR PREVENTING RETHROMBOSIS

If we accept the need for a therapeutic program which will both achieve rapid clot lysis and assure vascular patency, the question immediately arises as to the best means of accomplishing these dual goals. At the present time, two major approaches have been proposed. One calls for continued administration of the fibrinolytic agent itself (beyond the period of clot lysis) until the threat of a recurrence of thrombosis is likely to have subsided. This method is based on the concept that any new thrombus formed will promptly dissolve as long as an active fibrinolytic system is maintained in the circulating blood.1,2 The other approach is to follow or combine initial fibrinolytic therapy with the use of anticoagulant drugs.3,4

Prolonged fibrinolytic therapy with any of the

* From the Enzyme Research Laboratory, Department of Medicine, Georgetown University Medical School and the District of Columbia General Hospital, Washington, D. C.
† Present address: Head, Chest and Contagious Disease Division, U. S. Naval Hospital, Bethesda, Maryland.

currently available agents is associated with several practical and theoretic problems. For example, Johnson¹ has indicated that streptokinase must be administered intravenously for prolonged periods and the dosage carefully regulated by frequent laboratory tests if a recurrence of thrombosis is to be avoided. If the dosage is too large, endogenous plasminogen will be depleted and any thrombus formed subsequently will not be subject to dissolution by streptokinase. If the dosage of streptokinase is too small, lysis of the initial clot may not be achieved. Whether other fibrinolytic agents (such as plasmin itself or plasmin-activator combinations) will be subject to this same need for regulation of dosage remains to be demonstrated. Indeed, the question of dosage requirements for initial clot dissolution with the available agents is not yet adequately resolved. 8-8 What might constitute adequate prophylactic or maintenance dosage is even less clear. Prolonged or repetitive administration of these agents also raises toxic and antigenic considerations which require further investigation. At this juncture, therefore, our group believes that the established anticoagulant drugs offer the most satisfactory means for reducing the risk of a recurrence of thrombosis after initial clot dissolution has been induced by fibrinolytic therapy.9

REQUIREMENTS FOR COMBINATION THERAPY

To be justified, combination therapy in any field must satisfy at least three fundamental requirements: (1) the combination should have definite therapeutic advantages over the use of either agent alone; (2) the combined agents should not increase significantly the risk of toxicity; and (3) one drug should not interfere with the activity of the other. Let us examine how well fibrinolytic-anticoagulant therapy satisfies these requirements.

Therapeutic Advantages: At least three therapeutic advantages might be expected by supplementing fibrinolytic with anticoagulant therapy: (1) prevention of recurrence after initial clot dissolution; (2) limitation of thrombotic extension if initial fibrinolytic therapy is not totally successful; and (3) protection against thrombotic extension in those instances when the original occlusive process is not susceptible to attack by fibrinolytic agents.

Prevention of Recurrence: In assessing the possible value of anticoagulant drugs in preventing recurrence of thrombosis, we should attempt

to define those conditions which may favor this development. It is likely that the tendency toward a recurrence of thrombosis after initial fibrinolytic therapy is determined by two major factors: (1) the extent to which clot dissolution is achieved, and (2) the persistence of those factors which precipitated the original thrombotic episode. Considering first the extent of clot dissolution to be expected, we find ample reason to doubt that any fibrinolytic agent, however potent, will consistently remove all thrombotic material present in an involved vessel. In human thromboembolism we cannot determine precisely the duration of the thrombotic process. Our ability to date its onset is limited by our total reliance upon the appearance of clinical signs and symptoms. These may not become evident for hours to days after the thrombotic process has started. Since a thrombus is a dynamic, changing entity, its structure may vary considerably from one patient to another at the time fibrinolytic therapy is instituted.10 If we are fortunate, we will detect the thrombus early when all areas are "fresh" (i.e., dependent for stability upon a fibrin meshwork) and therefore susceptible to fibrinolytic attack. In many instances, however, we must expect that some areas of the thrombus will have altered their original fibrin structural support (or become endothelialized) and thereby have been rendered "resistant" to such drugs. Furthermore, even though the whole thrombus may be susceptible, total dissolution may not be achieved by the initial dosage schedule employed. Thus, even with an ideal fibrinolytic agent, we must accept the likelihood that some residual thrombus often may remain after fibrinolytic treatment to provide a nidus for recurrence.

Turning next to the question of the persistence of "thrombogenic factors," we again find cogent reasons for advising concomitant anticoagulant therapy. Investigations in experimental animals indicate that the likelihood of a recurrence of thrombosis is high in methods which produce thrombi by traumatizing the vessel wall1,10 but negligible in those technics which induce thrombosis by combining reversible vascular stasis with a transient hypercoagulable state.11,12 Transposing these observations to thromboembolic disease in man, we might suspect that transient stasis and hypercoagulability are responsible for most instances of postoperative venous thrombosis; therefore, anticoagulant protection after clot dissolution usually would be unnecessary. On the other hand, we might also suspect that such protection will be necessary in peripheral and visceral arterial thrombosis since these events usually are related to permanent atherosclerotic changes in the vessel wall. While such suspicions appear fully warranted, we currently have no means for verifying them in a given patient. Therefore, we believe that all patients treated with fibrinolytic agents should have the additional protection of anticoagulant therapy until reliable criteria permit us to differentiate between the "good risk" and "poor risk" subjects in terms of tendency toward recurrence.

Interval Protection: The second possible therapeutic advantage of combined therapy is "interval protection" against thrombotic extension if initial fibrinolytic therapy is not totally successful. Until dosage schedules are more fully developed, it is likely that instances of partial dissolution will occur even though the entire thrombus is susceptible to fibrinolytic attack. In such situations, anticoagulant drugs would block extension of the residual thrombus until fibrinolytic therapy could be renewed. Thus, the penalty for errors in initial dosage would be somewhat reduced.

Protection in "Resistant" Occlusions: Present diagnostic criteria do not permit accurate differentiation between the various causes of vascular obstruction, particularly in arterial occlusive disorders. Embolization of atherosclerotic plaque material or of thrombi which are no longer subject to fibrinolytic attack, spasm and subintimal hemorrhage may lead to the same clinical picture as produced by thrombosis per se. Studies with radiopaque media cannot define with precision which of these obstructive events has occurred. If the former mechanisms are operative, medical therapy can offer only protection against propagation of secondary thrombi. The anticoagulant drugs currently offer a more practical and predictable means for achieving this goal than the fibrinolytic agents.

TOXICITY

If two agents with an effect upon the hemostatic apparatus are to be employed simultaneously, we must have sound data indicating that the risk of toxicity, particularly hemorrhage, is not unduly enhanced. This requires knowledge of the dosage-response characteristics, the mechanism of action and toxicity of each drug. Such data can help us to predict not

IN VITRO STUDY

"potency" as defined by various assay systems

IN VITRO PLUS IN VIVO STUDY changes in coagulation factors changes in components of fibrinolytic mechanism

other "non-specific" effects (hematologic, hepatic, etc.)

IN VIVO STUDY ONLY

ability to promote lysis of thrombi "systemic toxicity" (fever, hypotension, nausea, etc.)

Fig. 1. Sequence of investigation required to establish characteristics of each type of fibrinolytic agent.

only which combinations will carry the least risk but also the optimum dosage of each drug.

During the past several years, a number of investigators have studied the laboratory and clinical alterations which follow intravenous administration in man of several plasmin, activator and combined plasmin-activator preparations. 1-5,8,13-17 These investigations have indicated that we cannot apply one set of rules to predict the effects of all fibrinolytic agents. The relationship between in vitro activity, changes in plasma coagulation and fibrinolytic components, toxicity and the achievement of thrombolysis in vivo differs for each preparation. For example, it is dangerous to conclude from data based on study of streptokinase that urokinase, plasminogen-streptokinase and plasminogen-urokinase preparations, spontaneously activated plasmin, etc., will behave in similar fashion either in vitro or in vivo.6 Such conclusions are subject to considerable question, for many studies1-5,8,10,13-17 have indicated that the characteristics of each fibrinolytic agent can be established only by a combination of in vitro and in vivo investigation, such as outlined in Figure 1. Therefore, we should not rely on specialized in vitro assays of "potency" to predict the effect of a given agent upon coagulation factors; nor can we determine the ability of all such drugs to achieve clot lysis by studying alterations in components of the fibrinolytic mechanism. With regard to assuring the safety of combination therapy this means that we must establish for each fibrinolytic agent the coagulation changes and toxicity which occur at a dosage that is effective in achieving clot lysis.

Guides to Safe Dosage of Fibrinolytic Agents: Certain rough guideposts are presently available regarding safe dosage with the fibrino-

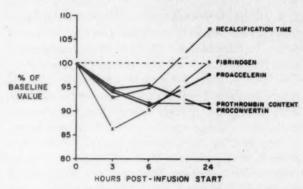


Fig. 2. Coagulation factor changes after intravenous infusion of an activator-plasmin preparation (Actase) in man.³ Note that fibrinogen is the factor influenced to greatest extent.

lytic compounds. With most agents tested thus far, the most striking effect upon the coagulation apparatus has been depression of fibrinogen, and it appears that decrease in fibrinogen will be the major limiting factor in dosage (Fig. 2). However, with each fibrinolytic agent (plasmin itself, different types of activators and activator-plasmin combinations) this relationship between reduction of fibrinogen and other phenomena may follow a different pattern. Furthermore, as initial dosage is increased, or with repeated administration, more profound or persistent changes may develop.

Safety of Anticoagulant Agent: It is evident that the safety of combination therapy will be related to both the type and the dosage of the fibrinolytic agent being used. This statement applies equally to the anticoagulant drug selected. Heparin would appear to have certain advantages over the prothrombinopenic agents in this regard. In addition to its probable superior antithrombotic activity, 18 heparin does not reduce the concentration of plasma coagulation factors. Its anticoagulant effect is based upon interference with the function of coagulation factors rather than upon their depression.19 Therefore, it will not exaggerate any abnormality of coagulation factors created by the fibrinolytic drugs. Furthermore, its effects can be quickly inhibited.20 Heparin also has the advantage of immediate onset of action, a quality of importance since a recurrence of thrombosis may develop rapidly after initial dissolution has been accomplished.1,4,19 The prothrombinopenic agents, on the other hand, could combine with the lytic agents in depressing specific plasma components to a critical degree, and both initiation and reversal of their effects require a longer time interval.

Antagonism Between Agents: One must establish that there is no antagonism in vivo between agents that are to be used concurrently. Von Kaulla²¹ has indicated that heparin may either depress or enhance fibrinolytic activity in certain in vitro systems, depending upon its concentration. Under what circumstances the effect of certain fibrinolytic agents may be altered by heparin in vivo requires further clarification. In our own studies in man¹² we have been unable thus far to demonstrate that therapeutic doses of heparin alter either parameters of plasma fibrinolytic activity or the clinical response induced by plasmin, activator or plasmin-activator combinations.

EXPERIENCE WITH COMBINED REGIMEN

Early in our clinical studies we employed short term fibrinolytic therapy alone in patients with acute thromboembolic disorders. In several instances of superficial venous thrombosis we noted that rapid disappearance of the thrombus was followed by recurrence of thrombosis within several hours. In deep thrombophlebitis and peripheral arterial thrombosis a similar sequence was observed in several patients. These experiences (and the theoretic considerations enumerated previously) soon led us to adopt a combined fibrinolyticanticoagulant program as our standard therapeutic regimen, a position also advocated recently by Ambrus and his associates.¹⁴

Clinical Method: Our usual procedure in therapeutic trials in human beings is to administer the fibrinolytic agent under study in a dosage which, on the basis of preliminary in vivo testing, has proved acceptable with regard to both toxicity and clinical response. Heparin therapy is begun intravenously or subcutaneously shortly after completion of the fibrinolytic infusion and is maintained thereafter whether or not additional fibrinolytic therapy is instituted. The standard dosage regimens of heparin we have used include 50 mg. administered intravenously every four hours; and 75 mg. every six hours, or 100 mg. every eight hours, subcutaneously, the latter schedules being most commonly employed. If fibrinolytic therapy is to be delayed for any reason, we do not hesitate to begin the administration of heparin in advance of the initial infusion. Indeed, our most recent experience indicates that there is little laboratory or clinical evidence to contraindicate the initiation of both forms of treatment simultaneously as long as the

coagulation effects which will follow the intended dose of the fibrinolytic agent have been

established previously.

The duration of anticoagulant therapy after initial fibrinolytic treatment is determined by a variety of factors including the specific disease process, the response to fibrinolytic therapy, and the patient's history of thrombotic events. ²² In situations in which it seems desirable to maintain an anticoagulant effect beyond several days, a prothrombinopenic drug is substituted for heparin after seventy-two to ninety-six hours.

Clinical Results: Our results with this regimen have been quite satisfactory from the point of view of both therapy and toxicity. Well over 200 patients with various types of thrombotic disease have been treated with heparin in conjunction with fibrinolytic agents of the activator and plasmin-activator types. Neither the incidence nor the degree of hemorrhagic complications has exceeded that expected with heparin alone despite steady increases in fibrinolytic dosage. Furthermore, we have observed few instances in which initial restoration of arterial or venous blood flow has been followed by the development of recurrent obstruction. In a controlled study of acute deep venous thrombophlebitis being carried out by our group9 forty-two patients now have been treated with a fibrinolytic-anticoagulant regimen. No recurrences have appeared within the two weeks after initiation of combined therapy.

COMMENTS

In examining current investigations dealing with the evaluation of fibrinolytic agents, we have been especially concerned that the importance of employing measures to insure the maintenance of vascular patency after clot dissolution has been achieved may not be generally appreciated. We must recognize that the patient is not protected against recurrence of thrombosis once the effect of the fibrinolytic agent has subsided. Under certain circumstances already discussed the likelihood of recurrence is probably high and thrombosis may reappear quite rapidly. Unless we have some means for distinguishing the original thrombus from the recurrent one, the door to interpretive error is opened. Such error is especially likely in trials in human beings, when we must often rely entirely upon clinical response as a criterion for clot dissolution and

lack adequate technics for differentiating "primary drug failure" from failure due to rapid return of thrombus. If clinical failure results from recurrence of thrombosis, inaccurate conclusions may be drawn regarding the efficacy and dosage of fibrinolytic agents. Therefore, we should neither equate fibrinolytic with anticoagulant therapy nor regard the two forms of therapy as mutually exclusive. Each approach may play a role in achieving optimum therapeutic results. The primary function of fibrinolytic agents is to promote rapid dissolution of the intravascular thrombus. The anticoagulant drugs continue to provide the best means for prompt, sustained and reliable protection against extension or recurrence of thrombosis.

The desire for definitive studies of the clotdissolving potential of various agents should not lead us to assume that we must use fibrinolytic therapy alone to provide reliable data. The natural reluctance of research workers to employ two agents concurrently should not obscure other factors which may make such a combined approach more meaningful than the use of one agent alone. Unless our experimental design permits assurance that recurrence of thrombosis is not an important consideration, we must have means of either detecting its development or preventing it. Prolonged fibrinolytic therapy or the addition of anticoagulant drugs to our regimen are the only means for accomplishing the latter. While an anticoagulantfibrinolytic combination currently seems the best choice, further study is required to define which approach will ultimately prove most acceptable in the management of thromboembolic disorders in man.

SUMMARY

The therapeutic goal in thromboembolism includes not only clot dissolution but also the maintenance of vascular patency. Clinical and experimental evidence, as well as theoretic considerations, suggest that a sequence of clot dissolution followed by a recurrence of thrombosis may occur when fibrinolytic therapy is relied upon exclusively. Concomitant use of anticoagulant drugs appears to offer the best current method for avoiding this sequence. Heparin, because of its mechanism of action, prompt onset of effect and easy reversibility, seems to provide the most desirable anticoagulant supplement to fibrinolytic therapy.

To insure that no increased hemorrhagic

risk attends this combined thrombolytic-anticoagulant program, the dosage-response characteristics of each type of fibrinolytic agent should be accurately defined. Evidence suggesting antagonism between thrombolytic and anticoagulant agents in vivo has not been encountered but several aspects of this problem require further study.

Failure to provide protection against a recurrence of thrombosis after initial fibrinolytic therapy can not only lead to misinterpretation of experimental data regarding the efficacy of fibrinolytic drugs but also may prevent attainment of optimum clinical results in man. While further development of fibrinolytic agents may obviate or alter the need for combination therapy, the concurrent use of fibrinolytic and anticoagulant drugs appears to provide the optimum therapeutic approach to thromboembolism at the present time.

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DISCUSSION OF PAPER BY DRS. MOSER AND HAJJAR

DR. RICHARD WARREN (Boston, Massachusetts): At what moment do you begin the anticoagulant treatment: immediately or after the fibrinolytic therapy?

DR. JULIAN AMBRUS (Buffalo, New York): Both Drs. Moser and Johnson pointed out that what we call plasmin therapy is a number of different things, depending not only on the preparation used but also on the amount of time over which the infusion is given. Prevention of reformation of clots and maintenance of patency of vessels after fibrinolysis should be the job of anticoagulant therapy and should not be expected from continued fibrinolytic therapy. Maintenance of patency of blood vessels after successful fibrinolysis should not be used as a criterion for evaluating fibrinolytic therapy. We would evaluate the data presented by Dr. Johnson in a different way than he has.

Concerning heparin, our clinical conclusions are the same as those of Dr. Moser. With Dr. Back we performed some carefully controlled studies in dogs. In these experiments we had evidence that heparin therapy applied simultaneously with plasmin therapy is superior to plasmin therapy alone.1 These differences, however, are too small to be demonstrable in the more crude clinical studies.

We have treated a number of patients with prepara-

tions which contained no activator activity whatsoever. Most of these were supplied to us by Drs. Kline and Fishman of Yale University. They activated plasminogen with streptokinase and then by various biochemical manipulations removed activator activity. We have also treated a very small number of patients with chloroform-activated bovine plasmin, winch has no activator activity at all. On the basis of these experiments we believe that this therapy does not produce more of a decrease in blood clotting factors than therapy with preparations containing activator activity. We have had comparable therapeutic results with a preparation having no activator activity, although our series is small.

Both Drs. Johnson and Moser alluded to the theory of endogenous activation, in that activator may activate small amounts of plasminogen trapped into the fibrin meshwork. We have investigated this theory in a carefully controlled study in animals.2 Venous clots were produced in dogs whose fibrinolysin system is activated only slightly and in guinea pigs whose system is not activated at all by streptokinase. Each animal carried clots of (1) human origin which, therefore, contain a large amount of endogenous activable plasminogen and (2) bovine origin, the plasminogen contaminant of which cannot be activated by streptokinase. If the theory of endogenous activation is correct, one would expect the human clots to dissolve following streptokinase therapy, while the bovine clots remain stable; in fact, none dissolved. When, however, either streptokinase or urokinase activated plasmin preparations were used, both types of clots dissolved with about the same speed. I do not deny that endogenous activation may exist. It probably is a factor, but I think it is a mistake to base therapy on the theory that this is the only important factor involved.

DR. JOHN WILSON (Hartford, Connecticut): Dr. Moser has referred to the persistence of factors which predispose to thrombosis. I wonder if he has noted any rebound phenomena following treatment with fibrinolysin which might tend to increase the coagulable state after treatment. I refer primarily to the rise in fibrinogen over the baseline or the lessening of the normal euglobulin activity.

DR. KENNETH M. Moser (Bethesda, Maryland): To comment on the questions that were raised by some

of the discussers: first we believed that until we had solved the controversy of how much heparin would create how much inhibitory effect, it was preferable to begin the administration of heparin promptly after the infusion of fibrinolysin had been completed. We have also more recently accumulated fairly extensive experience with simultaneous administration of the two, and I was glad to learn that Dr. Ambrus considers this at least not a bad situation because thus far we have not observed any difference between simultaneous dosage and dosage of heparin following administration of the fibrinolytic agents, either from the viewpoint of the particular in vitro tests that we perform or from the viewpoint of clinical response. Any of the many satisfactorily established dosages of heparin administered subcutaneously or intravenously are acceptable. We personally use a dosage schedule of either 75 mg. every six hours or 100 mg. every eight hours subcutaneously after starting with intravenous use.

I did not advocate in my paper that activation of plasminogen within a clot was the only method for achieving clot dissolution. I think that there is room for several theories at the present time and indeed, many of us, including our own group, are using both types of preparations (each having been analyzed prior to use for its activator and plasmin content) to try to bring some clarity to what is presently a rather clouded state of affairs. We are following this procedure because, as we indicated in our paper, we believe that reliance upon in vitro tests to establish whether a drug will achieve an in vivo effect is a rather dangerous procedure. Therefore, many of us, lacking confidence in such tests, believe it is necessary to carry out in vivo study of different types of agents, regardless of what our in vitro tests have shown.

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Dose Response Curves with Varying Doses of Fibrinolysin*

WILLIAM L. WILSON, M.D., FRANCIS NASO, M.D. and ELLEN LIPPMANN, B.S. Philadelphia, Pennsylvania

THE THEORY of the treatment of thromboembolic disease is undergoing a rather marked change. By logic the theory of dissolving clots rather than preventing the formation of clots, which is rather difficult, would appear to be good. However, the ideal method of accomplishing dissolution of clots has not as yet been determined. To some extent this is due to a lack of knowledge about the biochemical and physical mechanisms involved not only in clot dissolution but also in clot formation.

At present a number of substances are known which will induce fibrinolytic activity in the blood. These include a variety of compounds such as preparations of non-toxic fungi, streptokinase, nicotinic acid²—and urokinase. The only preparation which can be utilized to any great extent clinically is purified streptokinase or streptokinase-activated fibrinolysin. Which of these will prove the preparation of choice is still an open question.⁵

Our thought in working with the streptokinase-activated fibrinolysin containing streptokinase was to develop an optimal regimen of administration, using maximum dosage for an arterial embolus or thrombus. The work reported herein has been developed in the hope that we could arrive at a method of administration of fibrinolysin which would give maximum fibrinolytic activity, as demonstrated by our method of determining euglobulin lysis time,⁴ without producing harmful sequelae.

To accomplish this we used a multiple dose regimen, infusing the fibrinolysin over a onehour period with a six-hour period between administrations. This regimen has been continued in most cases for forty-eight hours when feasible.

MATERIAL AND METHODS

The patients in this study were hospitalized at

Hahnemann Hospital. The fibrinolysin† was mixed immediately prior to administration and was infused over a one-hour period under our supervision. All blood samples were collected and chilled to 4°c. If the laboratory procedures were to be performed within a short period of time, the samples were kept at this temperature; if not the plasma was separated and stored in the frozen state.

Fibrinogen determinations were performed by digestion of the fibrin clot and determination of the tyrosine content. The determination of euglobulin lysis times was carried out by a previously described method.⁴ In reporting the euglobulin lysis times, there is variation in the length of the control time. Based on our experience in other studies employing this method, we have selected the time of 360 minutes (six hours) as the shortest limit of normal.

RESULTS

Initially, we administered fibrinolysin in single doses to become familiar with the amount of activity to be expected and the effect on the fibrinogen levels and the toxicity.

Two patients received doses of 50,000 units. Both demonstrated increased fibrinolytic activity at the end of the fibrinolysin infusion (Table 1) and no untoward reactions were noted. Four patients then received 100,000 unit doses of fibrinolysin. Two patients demonstrated activity similar to that seen with the 50,000 unit doses; one had more activity and one, no activity. Again no untoward reactions were seen and no significant change occurred in the fibrinogen levels (Table 1). A total of six patients were then given 200,000 doses of fibrinolysin with the results shown in Table 1. With the dose of 200,000 units there was a more marked decrease in the euglobulin lysis time at sixty minutes. This occurred in five of the six patients. Only once did the 120-minute speci-

† Thrombolysin supplied by Merck Sharp & Dohme West Point, Pennsylvania.

^{*} From the Department of Internal Medicine, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania.

Table 1

Effect of Increasing Doses of Fibrinolysin on the Euglobulin Lysis Time and Fibrinogen Levels

Units No. of			Euglobulin Lysis Time (min.)					Fibrinogen Levels (mg./100 ml.)				
	Patients	0	60	120	180	240	0	60	120	180	240	300
50,000	1	300+	110	_		-	300	290	_			
,	2	300+	180	300+	_	_ '	310	290	310			
100,000	1 '	480+	140	_		_	_	_		_	-	
	2	360+	130		_	_	360	330	350			
	3	360+	80	360+	-	-	300	295	295			
	4	360+	360+	_	360+		290	290	_	290		
200,000	1	480+	30	150	480+	480+	290	240	260	_	200	165
	2	300+	120		360+	-	300	240	_	275	-	275
	3	360+	-70	_	360+		270	205	-	235	_	260
	4	420+	420+	420+	420+	_	360	360	350	360		
	5	420+	80	420+	-	420+	290	285	290	_	290	
	6	360+	35	300	_	_	340	330	330			

men show increased fibrinolytic activity. In one patient there was no effect on the euglobulin lysis time. At this level there was a significant decrease in the fibrinogen level in three of the patients. However, none of these were levels at which bleeding would be expected. We cannot explain the continual decrease in the fibrinogen level as well as the prolonged increased fibrinolytic activity.

To determine whether or not the effects of the intravenous administration were cumulative or constant, blood samples were withdrawn at timed intervals and the euglobulin lysis time determined. The results in three patients indicate that the maximum effect occurs shortly after the infusion is started and remains fairly constant throughout the period of the infusion (Table 11).

Effects of Repeated Doses of 200,000 Units of Fibrinolysin: After obtaining the previous data we adopted the regimen of 200,000 units of fibrinolysin every six hours for four to eight doses (Table III). Instead of indicating the serial euglobulin lysis times, only the specimen obtained when the infusion was discontinued is shown in the table. The fibringen levels in the first column are controls. The second column shows the lowest values obtained and the final fibrinogen level obtained four to six hours after the last administration of fibrinolysin. In the four patients and particularly in one patient (Case 2) in whom there was no increased activity after the first dose, there appears to be a somewhat cumulative effect with repeat doses even though there was no increased activity in any of the 120-minute secimens except once. One patient (Case 5) showed marked activity in all sixty-minute specimens. All fibrinogen levels demonstrated a significant drop. In three of the four patients in whom these values were checked, the final fibrinogen level was still less than the control level.

We had the opportunity to retreat one patient with fibrinolysin after a lapse of slightly over one month. His initial course of treatment was during the early phase of the program and the decision was made to attempt to administer 300,000 unit doses at six-hour intervals. After the second administration of 300,000 units, hematuria developed and further doses were not given. The increased fibrinolytic activity obtained after each of the doses was of longer duration than had been noted with multiple 200,000 unit doses. Whereas the changes in the fibrinogen levels following the first dose are not too striking, those following the second administration are quite profound. Fortunately,

Table II
Euglobulin Lysis Times in Minutes During Infusion of 200,000 Units of Fibrinolysin

Case	Level at:								
No.	0 min.	15 min.	30 min.	60 min					
1	420+		50	60					
2	420+	35		90					
3	420+	30	. 30	25					

Table III

Results of Repeat Administration of 200,000 Units of Fibrinolysin at Six-Hour Intervals

Case No.		Eu	globulin		mes at En	d of Infu	sion			1	nogen Lev g./100 ml.	
140.	0	1	2	3	4	5	6	7	8	Control	Lowest	Final
1	360+	120	240	20	30	20	70			300	205(4)*	285
2	420+	420+	210	150	180	100	50	60	20	360	260(8)	300
3	420+	80	20	20	15	-	-	-	_	290	220(4)	300
4	360+	70	30	20	20	-	_	-	-	270	165(2)	216
5	360+	35	20	60	50	30	10	15	-	_		

^{*} Numbers in parentheses indicate which administration it followed.

the patient recovered with no sequelae; approximately one month later the administration was repeated after the removal of a saddle embolus from the bifurcation of the aorta. A total of six doses of 200,000 units each and two doses of 300,000 units each were administered with the customary six-hour interval between doses. As shown in Table IV little activity was obtained and the effect on the fibrinogen levels was quite insignificant when compared to the previous values. It was our impression that antibodies to plasmin or streptokinase (or both) had developed in this patient and that this explained the lack of increased fibrinolytic activity.

TOXICITY

In previous studies with a similar agent we encountered rather severe toxicity, consisting of nausea and in some instances, vomiting, accompanied by a delayed increase in temperature to levels of 104°F. In one such instance the development of fever was accompanied by a

shaking chill. In the present series, we had no significant complications of this type. The only complications were the previously mentioned cases of hematuria and two other instances of abnormal bleeding. In both of the latter instances the patients were receiving coumarin derivatives and were considered well controlled. The first patient received three doses of fibrinolysin and then complained of severe headache. At first it was assumed that this was a side effect of the fibrinolysin administration which was discontinued approximately twelve hours later. When the patient still complained of a severe headache, a lumbar puncture was performed and grossly bloody spinal fluid was discovered. An arteriogram at a later date revealed no evidence of an intracranial aneurysm. There was no evidence of excessive fibrinolysis or a marked decrease in the fibrinogen level. In the second patient, the subarachnoid hemorrhage was less severe and there was a marked decrease in the fibrinogen level.

TABLE IV

Effect of Repeat Administration of Fibrinolysin to a Patient One Month After Previous Therapy with Fibrinolysin

Date and						Fibrinogen Levels (mg./100 ml.)								
Units Given	Control	60	120	180	240	300	360	Control	60	120	180	240	300	360
9/1/59					-									
300,000	390+	30		306		285	390+	480	450		450		345	425
300,000		25	100	360	390+			1	240	140	120	250		
10/5/59						1				0.00				
200,000 (1)	480+	250	480+		480+		480+	320	290	300		300		310
200,000 (2)		220	480+					310	220					000
200,000 (3)		300	480+					310	320			***	***	
200,000 (4)		240	480+		480+		480+		300	310		340		350
300,000 (5)		300	480+		480+		480+		220	230		260		240
300,000 (6)		330	480+		480+		480+		260	270		275		300
200,000 (7)		330	480+		480+		480÷		290	295		310		330
200,000 (8)		330	480+		480+	,	480+	1	260	285		300	300	315

SUMMARY

The intravenous administration of fibrinolysin to patients induces increased fibrinolytic activity as measured by the euglobulin lysis time. Except as noted doses of 200,000 units every six hours appear to be safe for use for periods up to forty-eight hours. Patients should be closely observed for bleeding and fibrinogen levels should be periodically checked. From our experience we conclude that it is probably not wise to use what we term maximum doses in patients who are receiving anticoagulants.

We are unable to explain the differences in response of individual patients; these will re-

quire further study.

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DISCUSSION OF PAPER BY DRS. WILSON, NASO AND LIPPMANN

Dr. James A. Evans (Boston, Massachusetts): Dr. Wilson, in a busy clinic in which it is impossible to carry out all these tests would it be possible to use the fibrinogen level alone as a guide to establishing a

safe dose of fibrinolysin?

DR. WILLIAM L. WILSON (Philadelphia, Pennsylvania): I believe that fibrinogen levels alone are probably not sufficient for evaluation and I do not know of any laboratory procedure that would be wholly adequate. Two of our patients had bleeding; one had fibrinogen levels below 120 mg./100 ml. of blood. The other had a severe subarachnoid hemorrhage when his fibrinogen level was 240 mg./100 ml. of blood. However, this patient was receiving Di-cumarol.® We have adopted the policy of determining the prothrombin time on the specimen taken immediately postinfusion and by observation of the clot that is formed, gross changes in fibrinogen levels can be seen. In this manner we have a much faster answer than if we had to wait for the determination of quantitative fibrinogen value. In one patient, who had a minimal subarachnoid hemorrhage, the technician notified us immediately that there might be difficulty because a poor clot formed in the onestage prothrombin test.

Experiences with Fibrinolysin in Peripheral Vascular Occlusive Disease*

WILLIAM G. ANLYAN, M.D., HUGO L. DEATON, M.D. and DONALD SILVER, M.D., WITH THE TECHNICAL ASSISTANCE OF JANET L. WEBSTER, M.T. (A.S.C.P.) AND ELEANOR K. SPADA, A.M.T.

Durham, North Carolina

The purpose of this paper is to present our experience with fibrinolysin (Thrombolysin†) in the treatment of twenty-three patients with thromboembolic disease. The historic background of the use of fibrinolytic agents and the biochemical derivation of the preparation used have been described previously. We agree completely with a recent editorial setting down the requirements for an ideal thrombolytic agent. The present study was initiated with a note of pessimism, since previous proteolytic enzymes administered by various routes had dismally failed in our experience.

MATERIAL AND METHODS

Twenty-three patients with various types of arterial and venous thromboembolic diseases are included (Table 1). The patients were divided into the following three groups: Group I: This includes our early experience when, because of limited knowledge at that time, inadequate doses were used. Group II: In this category, adequate doses were used, but objective evaluation of lytic effect was not possible; instead, subjective changes and alterations in blood clotting components were studied. Group III: In these patients, objective evaluation of the lytic effect on the diseased vessel as well as the changes in the blood clotting factors were possible.

The blood coagulation studies included the determination of (1) one-stage Quick prothrombin time; (2) specific prothrombin time; (3) factors v and vii; (4) partial thromboplastin time; (5) fibrinogen; (6) euglobulin clot lysis time; (7) Lee-White clotting time; (8) white blood cell count; (9) hematocrit; and (10) urinalysis. These studies were made just before treatment and at frequent intervals during the course of fibrinolysin administration.

In addition to the careful clinical history and

physical examination of the patient, accessory studies included phlebography and arteriography, whenever possible; oscillometry; photographs of the affected structures; measurement of the circumference of the limbs; repeated determinations of blood pressure and temperature throughout the period of study; and histologic evaluation of all surgical specimens.

RESULTS

Results in Group III: Of the nine patients in group III in whom objective evaluation was possible, six patients had fresh arterial thromboses of three to fifty hours' duration (Table II). The results in these six patients were judged to be "good" because of the return of pulses in the affected vessels and salvage of the

TABLE I
Twenty-Three Patients Treated with Fibrinolysin

Group I. Inadequate dosage (up to 100,000 units per 24 hours), early experience	3
Deep venous thrombosis	4
Superficial venous thrombosis	1
Saddle embolus	1
Axillary artery occlusion	1
Right common iliac artery occlusion	1
Femoral artery occlusion	1
Group II. Adequate dosage (over 300,000 units per 24 hours), but lack of objective criteria	T
Deep venous thrombosis	3
Cerebral embolus	1
Popliteal artery occlusion (intimal dissection)	1
Group III. Adequate dosage (over 300,000 units per 24 hours), with objective evaluation	,
Retinal artery thrombosis	1
Brachial artery embolus	1
Femoral artery occlusion	5
Multiple digital artery thromboses	1
Prophylactic following femoral arterial inti-	
mectomy	1

[†] Supplied by Merck Sharp & Dohme.

^{*} From the Department of Surgery, Duke University School of Medicine, Durham, North Carolina. This project was supported by Grant H2624 from the U. S. Public Health Service, by a grant-in-aid from Merck Sharp & Dohme, and the Duke University Gerontology Council.

Table II
Results of Treatment in Group III (Nine Patients)

Case No.	Age (yr.), Sex	Weight (kg.)	Diagnosis	Interval Between Onset of Occlusion and Onset	Daily Dose of Fibrino- lysin	Duration of Treatment		atus	Side Effects	Result
	Sex			of Treat- ment (hr.)	(units)	(days)	Before	After		
1	80, F	70	Retinal artery thrombosis	6	300,000 to 500,000	4	Occluded	Patent	Chills, fever and pain in the joints 24 hr. after stopping treatment	Good
2	43, F	67	Right femoral artery throm- bosis; carci- nomatosis of ovary	3	350,000	2	Pulseless right leg; cold	Return of all pulses ex- cept dorsalis pedis	None	Good
3	52, F	98	Femoral arterial thrombosis following en- darterectomy	50	350,000 to 475,000	3	Absent popliteal and pedal pulses	Return of popliteal pulse	None	Good
4	52, F	85	Femoral-pop- liteal throm- bosis follow- saddle embo- lus	3	300,000	3	Cold pulseless right leg	Return of right dor- salis pedis pulse; viable limb	None	Good
5	65, M	69	Right external iliac arterial occlusion; thrombectomy followed by	3	620,000	3	Absent femoral pulses	Return of fem- oral and pop- liteal pulse; resulted in much lower	None	Good
6	37, F	57	fibrinolysin Bilateral mul- tiple digital arterial thromboses	48	225,000 to 400,000	3	Gangrene of finger tips; ischemic dis- coloration of both hands	amputation Clearing of discoloration bilaterally	None	Fair
7	66, M	77	Femoral arterial thrombosis after throm- boendarterec- tomy	24	300,000 to 1,400,000	3	Absent popliteal and pedal pulses	Pulses returned for 1 day but throm- bosis reoc- curred 12	None	Fair
-/3								hr. after treatment was stopped		
8	55, M	86	Aneurysm of in- nominate ar- tery with em- bolus to right brachial artery	24	600,000	4	Pulseless right arm; cold	No change	Guaiac-posi- tive stool from pre- vious peptic ulcer	Poor
9	18, M	68	Traumatic arteriovenous fistula of femoral artery and vein; constricted femoral artery	4	150,000 to 200,000	2	Prophylactic treatment; pulses normal	Pulses normal	None	Good
			and intimal damage after repair	-12 A	*795	-545	- Prod			

limb or a significant portion thereof. Only one patient (Case 8) did not derive any benefit from treatment. He was seen with a pulseless cold arm twenty-four hours after an embolus entered the right brachial artery. Despite embolectomy followed by fibrinolysin therapy, there was no improvement and he underwent eventual amputation. Another patient (Case 7) had a thrombosis of the femoral artery after undergoing thromboendarterectomy for arterio-

sclerosis obliterans. Twenty-four hours postoperatively, pulses disappeared in that limb and fibrinolysin therapy was started. He received doses ranging from 300,000 to 1,400,000 units daily for three days with a return of all pulses. However, twelve hours after discontinuation of therapy, a recurrence of thrombosis occurred and amputation was performed. No result is tallied in the patient (Case 9) in whom fibrinolysin was used prophylactically

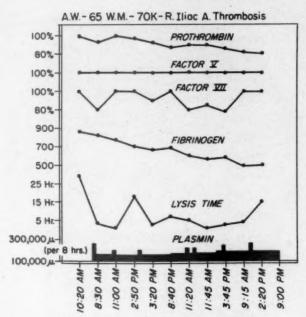


Fig. 1. Case 5. Coagulation studies.

after arterial trauma and repair; however, no thrombosis occurred in the damaged vessel postoperatively.

There were two patients (Cases 5 and 6) in whom the unusual finding of hyperfibrinogenemia was discovered in the course of our routine coagulation studies. Moderate improvement followed the administration of fibrinolysin; amputation was performed at a level considerably lower than had been anticipated before treatment.

CASE 5. This patient was found to have a hyperfibrinogenemia in the course of the routine studies. He was a sixty-five year old white man with a fresh thrombosis of the right external iliac artery superimposed on an underlying arteriosclerosis obliterans. Thrombectomy followed by fibrinolysin therapy resulted in the return and maintenance of arterial pulses including a bounding popliteal artery; this led to a lower amputation below the knee instead of the mid-thigh level. Fibrinogen level was 858 mg. per cent, initially, and 579 mg. per cent, thereafter. The euglobulin clot lysis time decreased from a pretreatment level of twenty-nine hours to a low of seventy-five minutes during the administration of fibrinolysin. Platelet count was in the normal range of 250,000 per cu. mm. throughout the period of study (Fig. 1).

CASE 6. This patient had a long history of small arterial and venular thromboses which had been classified as an "idiopathic vasculitis." She was seen on this occasion forty-eight hours after the onset of gangrene of several digits and a patchy, bluish discoloration of the skin of both hands (Fig. 2). Moderate improvement occurred within forty-eight hours after the administration of fibrinolysin therapy (Fig. 3). It was surprising to find on multiple determinations that plasma fibrinogen levels were consistently over 1,000 mg. per cent with a recheck in two separate laboratories. She had a coincidental increase in thrombocytes with levels ranging from 445,000 to 880,000 per cc. mm. Her clinical course to date has been one of progressive improvement and the fibrinogen levels have gradually decreased to a plateau of around 430 mg. per cent. A specimen of the digital arteries (Fig. 4) from her subsequent amputations of the finger tips revealed a concentric thickening of the intima compatible with the gradual deposition and organization of fibrin. It was also noteworthy that in the presence of the hyperfibrinogenemia, the usual dose of 225,000 to 400,000 units



Fig. 2. Case 6. Hyperfibrinogenemia and gangrene of finger tips before the administration of fibrinolysin.



Fig. 3. Case 6. After forty-eight hours of fibrinolysin therapy showing regression of avascular changes of the hands and limitation of gangrene to finger tips.

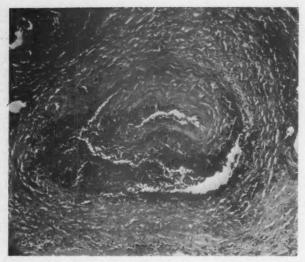


Fig. 4. Case 6. Section of digital arteries showing the thickening of the intima.

of fibrinolysin per day for three days had a lesser effect on the clot lysis time than in the other patients in the series. It decreased from thirty hours to only three hours (Fig. 5).

Bleeding Complications: Special mention should be made of the lack of bleeding problems when fibrinolysin was used in the immediate postoperative state in several of our patients.

CASE 2. A typical example is the patient in whom a right femoral arterial thrombosis occurred after pelvic perfusion with chemotherapeutic agents for ovarian intra-abdominal carcinomatosis; at the time of surgery, on three occasions the artery was reopened to evacuate a fresh thrombus. Immediately

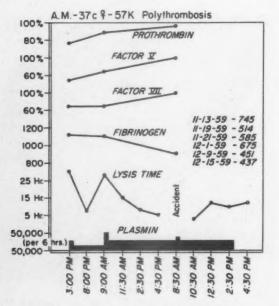


Fig. 5. Case 6. Coagulation studies.

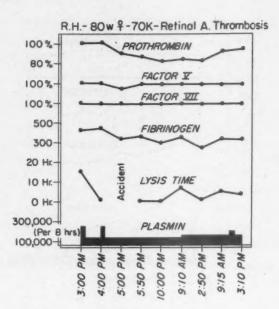


Fig. 6. Case 1. Coagulation studies.

after operation, the femoral arterial and distal arterial pulses disappeared again and fibrinolysin therapy was started after three hours. There was a return of all pulses in the limb except the dorsalis pedis within twenty-four hours with a dosage of 300,000 units of fibrinolysin per day for two days. There was no evidence of bleeding from the multiple surgical incisions including a right thoracotomy incision (used for temporary occlusion of the inferior vena cava) with catheter drainage. The euglobulin clot lysis time was decreased from a control of over twenty-four hours to two and a half hours; other coagulation studies revealed no significant alterations resulting from fibrinolysin therapy.

In the studies of blood coagulation factors of all patients, there were essentially no discernible changes in any of the determinations with fibrinolysin therapy except the fibrinogen levels and euglobulin clot lysis times. There was a slight reduction in plasma fibrinogen levels and a noted decrease in lysis times which appeared to represent the best measure of activity of the drug. A typical example of this is seen in the patient (Case 1) (Fig. 6) in whom one of the more dramatic lytic effects was noted. This patient arrived six hours after the sudden onset of blindness in the right eye. With fibrinolysin therapy, there was complete restoration of the retinal circulation within twenty four hours as witnessed by funduscopic examination and photography (Fig. 7).

Side Effects: The ill effects of the drug were mild and apparent in three patients only; one patient had a high spiking fever for two days, another had a slight rise in temperature to 38.5°

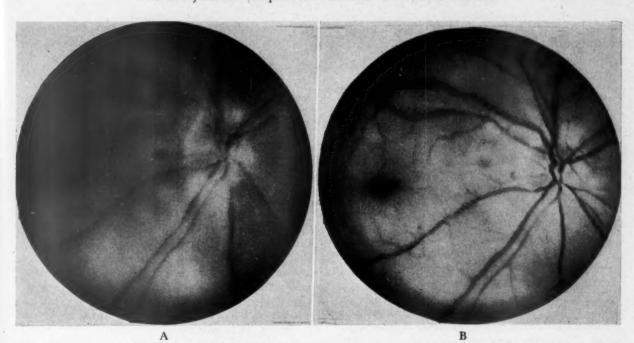


Fig. 7. Case 1. A, retinal arterial thrombosis of six hours' duration immediately before therapy. B, twenty-four hours after thrombolysin therapy for retinal arterial thrombosis.

g. without chills, and a third complained of arthralgia twenty-four hours after concluding the fibrinolysin therapy. The fever in the first patient was the only serious reaction. This could have been due to the release of bacterial products in the dissolution of a superficial thrombosis induced by intravenous therapy.

COMMENTS

Fibrinolysin therapy with the specific product, Thrombolysin, appears to be a definite asset in the treatment of thromboembolic disease. In the past, administration of anticoagulant drugs provided a means to prevent the propagation of thromboses; this preparation of fibrinolysin has been shown conclusively in these studies to promote the lysis of fresh clots in the vascular system. On the other hand, it was ineffective in the patient (Case 8) in whom the occlusion was due to an old organized embolus rather than a fresh thrombus. There are still many variables to be worked out, such as how long the fibrinolysin therapy should be continued. Should it be succeeded by routine anticoagulant therapy? What is its optimum role in combination with standard vascular surgical procedures such as embolectomy and thromboendarterectomy? From these studies, it appears to be safe to administer it in the immediate postoperative state without the threat of bleeding due to increased fibrinolytic activity.

In contrast to other proteolytic and fibrinolytic

preparations, the paucity of ill effects is most promising. The constancy of the dose required for optimal effect and the simplicity of laboratory control with euglobulin clot lysis times would be favorable in the promotion of the drug for use in smaller hospitals.

Of all preparations available to date, this drug is the most promising one, fulfilling the requirements of an ideal thrombolytic agent. However, its use does not preclude the rigid adherence to fundamental surgical principles. In surgical disorders, it should be regarded as an adjunct in therapy rather than as a substitute for time-proved surgical procedures such as embolectomy.

SUMMARY AND CONCLUSIONS

Twenty-three patients with thromboembolic disease have been treated with fibrinolysin therapy. Nine of these patients (group III) received adequate amounts of fibrinolysin, followed by objective means. Only one of these nine patients was not helped by the treatment with fibrinolysin. Two of the twenty-three patients had uncomplicated febrile responses while receiving fibrinolysin and one patient had a vague arthralgia twenty-four hours after concluding treatment. No other reactions of any sort, including bleeding tendencies, were noted.

It would appear that in adequate amounts,

fibrinolysin is a safe and useful agent for the treatment of thromboembolic diseases.

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Discussion of Paper by Drs. Anlyan, Deaton and Silver

DR. LESLIE MORRIS (Pittsburgh, Pennsylvania): Practicing clinical angiologists who view with some trepidation the many untoward reactions to fibrinolysin and activator substances continue to omit fibrinolysin in the treatment of patients with thromboembolic diseases. Our results have not been unsatisfactory. The demonstration that thrombi frequently may fracture or undergo spontaneous lysis when produced experimentally in the femoral artery of dogs, and the hazards which attend surgery during the critical twenty-four hours following an acute peripheral arterial occlusion prompted us to treat a number of patients so afflicted at the Montefiore Hospital, Pittsburgh, with intermittent intravenous administration of heparin utilizing the device first described by Sanford Wessler. The only limiting criterion for inclusion in the series was that a patient must not exhibit overt gangrene of any portion of the affected limb before receiving heparin. Incidentally, 68 per cent of our patients received the first dose of heparin within twelve hours of the occlusion. Our initial results have been encouraging; sixteen consecutive patients with major arterial occlusions involving the extremities have received this form of therapy during the past three years. One patient died from the effects of warfarin three months after being discharged from the hospital. There were four patients in whom either the external iliac or the femoral artery was involved and treatment with heparin was started within three hours of the occlusion. In three of these a persistent return of popliteal and dorsalis pedis pulses was noted. As a control we checked the results of all peripheral arterial embolectomies performed at the Montefiore Hospital during the same three-year period. Seven embolectomies were performed; in four of these cases the patients died following surgery. It is

germane to point out that the three survivors all received intermittent intravenous administration of heparin following surgery. One of these patients died from a cerebral hemorrhage six months later, the other two are doing well. Follow-up studies in our series performed in January 1960 revealed that two of the patients had died: one as described previously and the second, an eighty-two year old woman who died of a myocardial infarction eighteen months after the acute peripheral arterial occlusion. The others were ambulant. The two patients with axillary arterial emboli of the upper extremities had regained excellent radial pulsations.

DR. FRANK GILBERTSON (New York, New York): The possibilities of using fibrinolytic agents as an aid in embolectomy have been touched upon. We have been using this material following removal of the embolus from a vessel hoping to aid in dissolving the peripheral propagated fresh thrombus. I would like to present the case of a forty-four year old woman who had acute femoral arterial occlusion with profound ischemia of the extremity. An arteriogram was taken showing the common femoral artery filling by collateral vessels, but the actual bifurcation was completely occluded. At operation the embolus was removed. However, the distal thrombus was incompletely extracted and another arteriogram was taken. The thrombus lay in the popliteal vessel with bypass collateral vessels filling the distal arteries. This woman had received 100,000 units of fibrinolysin intravenously preoperatively and 200,000 units were administered intra-arterially. No further surgical procedure was performed since the extremity had improved following removal of the embolus from the femoral artery. A few days later, pulses returned and two weeks later an arteriogram revealed the popliteal artery open and what had appeared to be a rather obvious and complete occlusion with a thrombus was now clear. This certainly was not a picture of recanalization but of a normal appearing artery.

DR. WILLIAM G. ANLYAN (Durham, North Carolina): Regarding Dr. Gilbertson's case report, I should like to congratulate him on a very nice result. With regard to Dr. Morris' note of dissidence I would say that heparin is good but not good enough. I would agree with the previous speakers that we need something to dissolve clots as well as to keep the clot from propagating. Furthermore, in arterial embolism, since most of these emboli are old organized fragments of thrombi, we believe that the surgical procedure is necessary to extract this old clot and that the fibrinolysin is of benefit in the dissolution of the propagated fresh thrombus. To question your confidence in the exclusive use of heparin in the treatment of patients with thromboembolic disease, I would like to say that we have recently reported on (Arch. Surg., 80: 105, 1960) twelve patients who had recurrent pulmonary embolism while they were receiving adequate doses of heparin.

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Treatment of Thrombophlebitis with a Fibrinolytic Agent

With Special References to Side Effects*

NANCY D. DAVIS, M.D., BRUCE J. CARROLL, M.D. and SAMUEL H. SCHWARTZ, M.D., F.A.C.G.

Plainfield, New Jersey

This report concerns our experience in treating fifty cases of thrombophlebitis with fibrinolysin. This material was used in a total of seventy-one patients, twenty-one of whom were treated for diseases other than thrombophlebitis. Data regarding side effects and laboratory studies have been included from both groups in order to obtain a larger number of observations regarding possible untoward effects of the drug.

METHODS

Case Selection: Our selection was made from patients admitted to Muhlenberg Hospital with diagnoses of thrombotic disease states and referred to us for fibrinolytic therapy by the attending physicians. The criteria for deep venous thrombosis were unilateral edema, which was not always measurable, and local tenderness or positive Homans' sign. Superficial thrombophlebitis was judged to be present when there was objective evidence of inflammation and tender venous cords were palpable. Patients with symptoms of less than ten days' duration were accepted as acute. Subacute cases were of ten to twenty-one days' duration. Although most of the patients were treated within seven days of the onset of symptoms, a few patients' symptoms were of longer duration.

Unfortunately, it was not feasible to set up an untreated control group.

Dosage and Administration: Fibrinolytic therapy consisted of one to ten intravenous infusions of 50,000 to 100,000 Merck units of human fibrinolysin dissolved immediately before use in 100 to 250 ml. of 5 per cent glucose in water. Infusions were administered under observation over a half- to two-hour period each and, when repeated, were given five to twelve hours apart. Rectal temperature and blood pressure measurements were frequently taken before, during and following each infusion for a period of twenty-four hours. Daily clinical evaluation and measurements of the leg were made by the same ob-

server according to a prescribed routine. Adjunctive anticoagulant therapy consisting of heparin and bishydroxycoumarin was administered in the majority of cases of deep thrombophlebitis.

Laboratory Determinations: Subject to the availability of the services of our routine clinical laboratory, determinations of total white blood cell count with differential count, blood urea nitrogen, urinalysis, and selected tests of liver function were made. Samples of citrated blood were centrifuged and the separated plasma was quick-frozen. Fibrinolytic activity was determined subsequently by the euglobulin clot lysis method.† Electrocardiograms were recorded before and after therapy in thirty-one patients.

RESULTS

Clinical Evaluation: Of the fifty cases (Table 1) of thrombophlebitis, forty-one were considered to be acute deep, four acute superficial, and five subacute deep and superficial. The average time required for measurements of the affected leg to become maximally reduced was 3.3 days, and for local tenderness to subside was 3.8 days. Average duration of bed rest was 9.9 days, and that of hospitalization was 10.5 days. It is of interest that seven patients with acute deep thrombophlebitis had evidence of probable pulmonary embolism or actual pulmonary infarction on admission. In one of these patients (Case 30) what was apparently a second and mild episode of pulmonary embolism developed ten days after fibrinolytic therapy and eight days after the thrombophlebitis had subsided. In another patient (Case 50) with acute deep and superficial phlebitis, who had been treated with anticoagulants, a recurrence of superficial

† Courtesy of Merck Sharp & Dohme Research Laboratories.

^{*} From the Departments of Medicine and Peripheral Vascular Disease, Muhlenberg Hospital, Plainfield, New Jersey.

TABLE 1 Clinical Evaluation of Treatment of Fifty Patients

	* The state of the		Duration	No. of Infu-	Dura- tion	Dura- tion	Maximal Dec Leg Measure		Subsidence	Ant
No.	Type of Thrombophlebitis	Associated Diagnosis	of Symptoms (days)	sions (100,000 units each)	of Bed Rest (days)	of Hospi- talization (days)	Clinical Measurements (inches)*	Time (days)	of Local Symptoms (days)	lan Use
1	Acute deep		2	1	9	13	F1/2, A1/2	3	3	Ye
2	Acute deep		1/2	1	4	9	C1	3	3	No
3	Acute superficial		1	1		8	ND	* *	2	No
4	Acute deep		7	1	7	9	C1	3	3	Ye
5	Acute deep	Probable pulmonary infarction		1	16	16	ND		5	Ye
6	Acute deep	Fractured femur	4	1	+	72‡	ND		Complicated by sepsis	Ye
7	Acute deep		4	3	3	8	F1, C3, T21/2	1	3	No
9	Acute deep		7	1	11	14	C1/2	2	3	Ye
10	Acute deep		4	2	5 ,	9	Ci	2	2	Ye
13	Acute deep		1	2	7	9	A3/8	1	2	N
14	Acute deep		5	2	7	9	F1, A1, C1/2	4	4	N
15	Acute superficial		6	2	10	15	NC		4	Ye
16	Acute deep	21	1	2 §	8 ,	12	F8/4	1	5	Ye
17	Acute deep	Fractured femur	5	1	#	17	C11/4, T11/4	3	1	Ye
20	Acute deep		1	2	10	14	F1/2	2	3	Y
23	Acute deep		7	2	6	7	F1/2, A1/2	2	2	Y
25	Acute deep		8	2	6	8	T1	3	3	N
26	Acute deep		1	4	22	24	F1/2, A1/2	3	5	N
27	Acute deep		. 7	2	9	13	NC		2	N
28 30	Acute superficial Acute deep	Possible pulmonary embolism	2 4	10	21	14 27	NC T3	5	5 5	N
33	Acute deep	embolism	7	3	10	12	F1/2	2	2	Y
34	Subacute deep		14	4	11	12	ND		3	Y
35	Acute deep	Probable pulmonary embolism	7	2	9	14	ND		4	N
36	Acute deep	Possible pulmonary embolism	7	2	9	13	NC		2	Y
37	Subacute deep and superficial	Probable pulmonary embolism	10	1	9	12	ND		13	N
39	Acute deep	Possible pulmonary embolism	3	6	13	18	F1, A1, C21/4	5	5	Y
40	Acute deep		1	4	10	11	ND		4	N
44	Acute deep		3	4	13	15	NC		5	Y
45	Acute deep		5	3	6	8	·C1/2, T11/2	2	4	Y
47	Acute deep		4	3	6	9	F1/2, A2/4, C11/4,	8	6	Y
40							T11/4	0		**
49	Acute deep	T	5	4	8	11	A1/2	2	3	Y
50	Acute deep and superficial	Fractured pelvis	5	5	†	20	ND		6	Y
51	Acute deep		3	2	14	14	F ¹ / ₂ , A1, C1, T ³ / ₄	7	4	Y
52	Subacute superficial		21	3	4	8	C1/2	2	9	N
54	Acute deep Acute deep		7	3 4	10	13 14	F ¹ / ₂ , A1 ¹ / ₂ A1, C1 ¹ / ₄ , T3	13	7	Y
57	Acute deep	Fractured hip	7	-4	6	10	A1/2, C1, T1/2	3	4	Y
58	Acute deep	Recent myocardial infarction	3/4	4	9	13	C3/4	2	5	Y
59	Acute deep	imarction	7	4	9	12	C1/2	2	2	Y
60	Acute superficial		2	4	10	14	NC NC		3	N
62	Acute deep		6	4	6	11	C1/2, T1/2	1	4	Y
63	Acute superficial and deep		3	4	8	10	A11/4, C1	3	4	Y
64	Subacute deep		10	4	17	21	A ¹ /2, C1 ¹ /2, T1 ¹ /2	7	5	Y
67	Subacute deep		14	6	14	20	A1, C3, T11/9	3	4	Y
68	Acute deep		3	6	16	20	NC		6	Y
69	Acute deep		1	3	14	16	NC		4	Y
70	Acute deep		5	4	9	12	A11/2, C11/4	4	2	N
71	Acute deep	Pulmonary infarction	1	4	18	18	ND		4	N
	Acute deep	1	2	4	7	10	NC		4	Y

* In the clinical measurements, F = foot; A = ankle, C = calf; T = thigh; NC = no change; ND = not determined. † Bed rest prolonged due to fracture. ‡ Excluded from average because hospitalization was prolonged due to fracture. § Infusions of 50,000 units each.

TABLE II Comparison of Results

Clinical Data	220000	Treated with Fibrinolysin					
	Present Series (50 patients)	Moser ⁶ (32 patients)	Moser ⁶ (30 patients)				
Average duration of bed rest (days) Average duration	9.9	7.7	10.8				
of hospitaliza- tion (days) Resolution of	10.5	14.8	19.4				
pain (days) Maximum reduc-	3.8	4.6	8.1				
tion in extrem- ity size (days)	3.3	4.8	. 7.2				

phlebitis occurred ten days after cessation of therapy. Average depression of prothrombin time was, however, only 57 per cent of control during this period.

Side Effects: Under our observation, a total of 218 separate infusions were administered to seventy-one patients. The most common side effect was hyperpyrexia (Table III). Twenty-one patients (29 per cent) were noted to have a rise in temperature of 2°F. or greater above baseline level. Temperature returned to normal within twenty-four hours in most cases. No prophylaxis or therapy for fever was given. Shaking chills occurred in four patients (6 per cent).

Hypotension occurred in one patient, with a drop in blood pressure from his usual hypertensive level of 160/80 mm. Hg to a low of 104/ 62 mm. Hg beginning eight and one-half hours after completion of the infusion and lasting sixteen hours. The period of relative hypotension began with a high temperature of 105° F., and pain in the lower part of the abdomen. Inasmuch as the patient showed no evidence of shock or tachycardia no vasopressor agent was given, and the blood pressure returned to normal. Two other hypertensive patients had transient drops in blood pressure, 160/100 to 140/70 mm. Hg and 170/100 to 140/80 mm. Hg, which were of doubtful significance in view of subsequent marked fluctuation of blood pressure. With variable personnel taking blood pressures

variations up to 20/10 mm. Hg are frequent, and therefore, we considered only changes greater than this to be significant.

Other side effects were nausea and vomiting in five patients, headache in five, flushing of the face in three, and pain in the back, chest or abdomen, two each. Hemorrhage did not occur.

The follow-up period of these seventy-one patients varies from eight months (in the earlier patients) to several weeks. So far, there has been no evidence of the development of homologous serum jaundice.

Laboratory Determinations: Total white blood cell and differential counts, carried out before and after therapy in fifteen patients, showed no appreciable change. Results of follow-up urinalysis in fifteen patients were within normal limits, except for one patient in whom a trace of albumin was reported without cellular elements. Follow-up blood urea nitrogen determinations in ten patients were within normal limits, except for one patient with a rise from 16.8 to 32.2 mg. per cent with normal urine findings. Follow-up tests of liver function in ten patients, including bilirubin, thymol turbidity and cephalin floculation, showed no detectable evidence of hepatic damage.

Fibrinolytic Activity: Blood specimens were drawn at the beginning, at the termination, and one hour after the termination of forty-two separate infusions in twenty-four patients. Measurable fibrinolytic activity (euglobulin clot lysis time of four hours or less) was noted in twenty-two instances and in seventeen patients. Because the number of patients showing fibrinolytic activity is small, no accurate correlation can be made between activity and clinical effects or pyrogenic effects, but grossly, no correlation appears to exist.

Electrocardiographic Studies: Of the thirty-one patients in whom electrocardiograms were taken before and after fibrinolytic therapy, twenty-five of the follow-up tracings were taken within twenty-four hours following completion of the last infusion, and the remainder within a four-day period. In eleven patients, electrocardiograms were taken while an infusion was in progress

In five instances, comparison of tracings showed significant change. In three (Cases 35, 37 and 41), initial tracings showed non-specific T wave changes and follow-up tracings showed changes compatible with pulmonary embolism. All three patients were admitted

with symptoms strongly suggestive of pulmonary embolism. A fourth patient (Case 61) with known rheumatic mitral stenosis and insufficiency and chronic auricular fibrillation had three episodes of peripheral arterial embolization and one of pulmonary embolization during her hospital course. In this patient, the post-treatment tracing was compatible with pulmonary embolism thought to be present at that time. The fifth patient (Case 54) was a seventy-five year old man whose initial tracing showed T wave changes of myocardial ischemia. A repeat tracing taken two days after completion of therapy showed slight increase of T wave inversion in leads 1, 11, V5 and V6 compatible with increased myocardial ischemia.

It is of interest that of the thirty-one patients who had serial tracings done, twenty-four were fifty years of age or older, and of these, seventeen had significant hypertension or demonstrable coronary artery disease. It is with this type of patient that the greatest incidence of change would be expected if fibrinolysin caused myocardial damage. However, only one patient exhibited such a change, and this may have been coincidental.

COMMENTS

There are excellent summaries giving the theoretical consideration of fibrinolytic agents and their clinical applications.

1 In vivo clot lysis with fibrinolysin has been unequivocally demonstrated. It remains for the clinician to prescribe dosage, determine efficacy and establish the safety of fibrinolytic therapy for routine clinical use in thromboembolic states.

Regarding dosage, we arbitrarily chose a scheme of intermittent therapy. Inasmuch as total dosage varied from 100,000 to 1,000,000 Merck units in our relatively small series of fifty patients, we cannot draw valid conclusions regarding optimum dosage levels. Determination of post-treatment plasma fibrinolytic activity affords no assistance if, as has been stated, in vivo lysis of vascular clots shows no correlation with plasma fibrinolytic levels.

Returning to clinical evaluation as a determinant of efficacy, we are hampered in our series because of a lack of an untreated control group. It is helpful to compare our statistics with those of Moser et al.⁶ who reported on a series of thirty-two patients with acute deep thrombophlebitis treated with fibrinolysin and anticoagulants, as compared to a control group of thirty patients treated with anti-

Table III
Comparison of Febrile Reactions

Series	Maximum Temperature Elevation (degrees Fahrenheit)						
	2-3	3-4	>4				
Present	11	6	4				
Moser ⁶	9	9	3				

Note: Twenty-one of seventy-one patients in our series had febrile reaction (29 per cent) as compared to twenty-one of sixty-three patients in Moser's series (33 per cent).

coagulants alone (Table II). We find that our figures for duration of hospitalization, subsidence of local pain, and reduction in measurements of the leg compare favorably, while the duration of bed rest was longer in our series. To these figures must be added our subjective clinical impression that our patients were appreciably helped.

The observed side effects were not serious. Hyperpyrexia was frequently encountered, as noted by other workers. Table III compares the incidence of fever in Moser's series with our own, and shows striking similarity. If severe systemic reactions with marked hypotension as reported were common, we should hesitate to use this drug. However, only one instance of significant hypotension was encountered.

SUMMARY

- 1. Fibrinolysin was administered to seventy-one patients in 218 separate intravenous infusions. Each infusion contained 50,000 to 100,000 Merck units dissolved in 100 to 250 ml. of 5 per cent glucose in water and was given over a half- to two-hour period. Side effects were limited to hyperpyrexia of 2°F. or greater in twenty-one patients, asymptomatic hypotension in three patients, symptomatic hypotension in two patients, nausea and vomiting in five patients, facial flushing in three patients, shaking chills in four patients, and pain in the back, chest or abdomen in two patients each. No serious side effects were encountered.
- 2. Fifty patients were treated for acute to subacute thrombophlebitis of the lower extremities. Average duration of bed rest was 9.9 days, and duration of hospitalization was 10.5 days. Measurements of the leg showed maximal reduction in 3.3 days. Local tenderness subsided in 3.8 days.

3. In spite of the high incidence of hyperpyrexia, we believe that fibrinolysin is a safe and beneficial drug for the therapy of deep thrombophlebitis. However, the protean nature of this disease makes clinical evaluation difficult.

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DISCUSSION OF PAPER BY DRS. DAVIS, CARROLL AND SCHWARTZ

DR. ERWIN DEUTSCH (Vienna, Austria): I would like to comment on another side effect of this therapy. In our routine scheme of treatment with streptokinase a priming dose is given (calculated from the streptokinase resistance test). During the first two hours we infuse two-thirds of this dose hourly and then half of it hourly. The thrombin time increases slowly at first and as soon as we have found this increase we reduce the dosage of streptokinase. Then the thrombin time remains at the same level but later it increases further. This increase could be an unexpected and unfortunate side effect, but in our opinion it is the opposite. We consider this side effect agreeable because a prolongation of the thrombin time from fifteen seconds to thirty and forty seconds is the same effect usually obtained with effective heparin therapy. We therefore believe that a new thrombosis will not occur during the first hours after the infusion of streptokinase is discontinued. This prolongation of the prothrombin time lasts about twenty-four hours. In the meantime we administer anticoagulants to insure that there is no recurrence of the thrombosis.

DR. N. BACK (Buffalo, New York): Dr. Davis and other speakers have indicated that intravenous administration of plasmin induces hypotension. It is of general pharmacologic interest to determine the mechanism of this hypotension. Dr. Guest, studying this phenomenon, successfully isolated a hypotensive

factor from bovine plasminogen preparations which he euphoniously termed vascularin.

We have studied this hypotensive reaction in dogs in our laboratory. Plasminogen preparations, per se, when injected rapidly into dogs, were found not to cause hypotension. Streptokinase, also, did not produce hypotension. However, rapid injection of streptokinase-activated human plasminogen (30 units/kg.) did cause a severe blood pressure fall. The latter is accompanied by a drop in fibrinogen levels. If, after the blood pressure returns to its preinjection level, another plasmin dose is administered, the blood pressure response diminishes. Repeated administration of plasmin within a short time interval will result in the complete abolition of the hypotensive response. This is a classic picture of tachyphylaxis. In this same dog we find that this resistance is maintained, to a large extent, for twenty-four hours. Injection of plasmin on the second day causes only a slight drop in blood pressure, and tachyphylaxis develops within a much shorter time period. This phenomenon is at present under investigation.

Of greater interest, perhaps, is the possible mechanism by which plasmin induces hypotension. We have excluded acetylcholine or histamine release by showing, as have Dr. Guest and his co-workers, that hypotension still occurs in animals pretreated by administration of atropine or antihistamines. Perhaps the kallikrein-kallidin vasodilator polypeptide system can be implicated, since plasmin may act on a precursor kallikreininogen, releasing kallikrein. Kallikrein, believed to be hypotensive per se, activates kallidinogen, bradykininogen or other protein precursors present in plasma, serum or tissues to form kallidin, bradykinin or other vasodilator polypeptide kinins. Louis and co-workers in England have in vitro evidence demonstrating the formation of vasodilator polypeptides when plasmin is incubated with plasma proteins.

DR. LESLIE MORRIS (Pittsburgh, Pennsylvania): Dr. Davis has stated that she has no controls. I would like to offer a series of ninety-two patients with venous thrombosis treated at the Montefiore Hospital, Pittsburgh during the past five years as a possible control series since none of these patients received fibrinolytic agents. Treatment was with intermittent intravenous administration of heparin. Often a prothrombinopenic agent was added to the regimen but without reduction of heparin dosage. All these patients had deep venous thrombosis. There were twenty-five pulmonary infarctions, twenty-two of which had occurred prior to the institution of heparin therapy; three developed during treatment. There were no deaths. On an average, pain had disappeared in the affected extremity within twenty-four hours after heparin administration was started. In fact, we had several patients in whom pain was absent within twelve hours. Tenderness upon palpation disappeared within an average of four days: mensuration

showed return to normal within five days. During the past twelve months the average duration of hospital stay was seven days for patients with calf vein thrombosis and nine days when the involvement was iliofemoral. We encountered no pyrexial reactions due to the administration of heparin and the total cost to the patient was, we believe, considerably less than when fibrinolytic agents are employed.

DR. NANCY D. DAVIS (Plainfield, New Jersey): Re-

garding the control series, it is very difficult to transpose data from one place to another. Evaluation must always be subjective. In our cases we were most rigid in evaluating the disappearance of the subjective complaints of the patient. In the future we hope to have our control series within our own institution. However, we had difficulty because our clinicians were so enthusiastic about this therapy, they were unwilling to cooperate in establishing a control series.



Effect of Fibrinolytic (Plasmin) Therapy on the Physiopathology of Myocardial Infarction*

PAUL RUEGSEGGER, M.D., IRWIN NYDICK, M.D., RAMON ABARQUEZ, M.D., FRED REICHEL, M.D., EUGENE E. CLIFFTON, M.D. and JOHN S. LADUE, M.D., F.A.C.C.

New York, New York

PIBRINOLYTIC therapy of coronary thrombosis, in contrast to peripheral thrombosis, presents two serious problems: the absence of fresh thrombi in 40 per cent of clinically diagnosed myocardial infarctions and the short viability of the blood-starved heart muscle, which might

render this treatment ineffective or dangerous. Our studies of the fibrinolytic effect upon coronary thrombosis and infarction were therefore extended to problems related to the mechanism of prenecrotic ischemic injury and extension of myocardial viability.

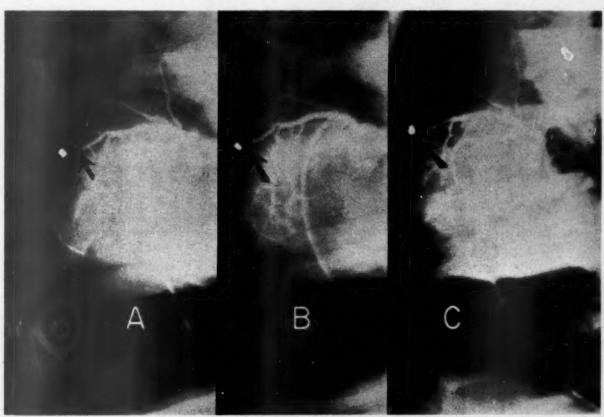


Fig. 1. Serial coronary arteriograms depicting the lysis of a coronary thrombus by systemic plasmin treatment. A, arteriographic filling defect (between arrow point and metal marker) indicating size of thrombus untreated for eight hours. B, partial lysis. C, complete lysis after four and one-half hours of intravenous plasmin therapy (4,000 units/kg./hr.). Patency of coronary arteries appears restored. The small residual filling defect is caused by the stenosing ligature. (Figures 1, 2, 5, 6 and 7 from: Ruegsegger, P., Nydick, I., Hutter, R., Freiman, A. H., Bang, N. U., Cliffton, E. E. and Ladue, J. S. Circulation, 19:7, 1959.1)

^{*} From the Clotting Mechanisms Section of the Division of Experimental Surgery and Physiology, Sloan-Kettering Division of Cornell University Medical College and the Department of Cardiology, Memorial Center, New York, New York. This work was supported by Grants H-2867 and C-2009, from the National Institutes of Health.

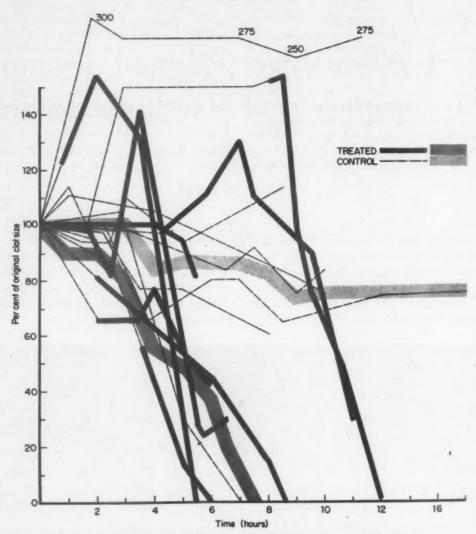


Fig. 2. Length of coronary thrombi in eight plasmin-treated and eight control animals. Clot size is indicated by measured length of filling defects in serial arteriograms. The curves show the relative variation of filling defects during control periods (broken lines) and during plasmin treatment (solid lines).

DEMONSTRATION OF CLOT LYSIS

Initial studies¹ convinced us that it is possible to dissolve coronary thrombi without harmful side effects and with a speed similar to peripheral clots. Thrombi were produced by local injections of a mixture of blood and serum into an isolated coronary artery. The coronary arterial tree, including the obstructed vessel, was delineated by serial arteriography. The process of lysis of an eight-hour old thrombus is illustrated by the series of arteriograms in Figure 1. The clot size, as indicated by the arteriographic filling defect in A, did not change throughout the entire control period. The obstructed artery became patent after four and a half hours of intravenous plasmin therapy as shown in C.

Such objective evidence of lysis was obtained in all dogs with significant fibrinolytic activity (fifteen to sixty minutes lysis time) in the blood stream.¹ Figure 2 shows the variations of the arteriographically determined size of the clot during treatment and control periods. In seven control animals the clots shortened slightly within twelve to fifteen hours probably because of retraction and minimal spontaneous lysis. The coronary thrombi in eight treated animals shortened much more rapidly and disappeared after three to seven hours. In one control animal with spontaneous fibrinolytic activity the same rapid dissolution of the thrombus was observed in the bloodstream (fifteen minutes lysis time).

FACTORS AFFECTING VIABILITY OF MYOCARDIUM

This dissolution time (three to seven hours) far exceeds the established time limit for toler-

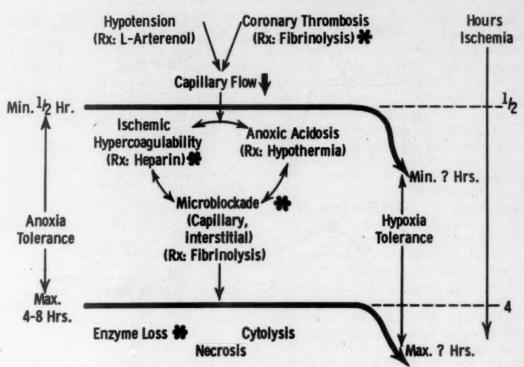


Fig. 3. Physiopathology of infarction. See text.

ance of anoxia (thirty minutes) after which necrotic death of heart muscle is supposed to be inevitable.^{2,3} This time honored belief about the critical importance of the time element is based on studies of infarcts in the normothermic dog with a non-lytic blood system of normal coagulability. But there must be other factors which govern the time limit of viability, because the entire heart can tolerate circulatory standstill up to four hours under hypothermia⁴ and with heparin perfusion.⁵ These observations presented the intriguing possibility that muscle breakdown may actually represent starvation necrosis and be related to microclotting induced by the products of anoxic metabolism.

Actually, we wondered from the beginning of our studies in 1956, why the viability of the heart muscle should be lost after only thirty minutes of anoxia, whereas muscular necrosis with cytolysis and enzyme loss is not manifest for four to eight hours. Figure 3 shows our concept of the physiopathology of this nebulous prenecrotic phase of infarction. Numerous studies resulted from this hypothesis (indicated by asterisks) the results of which will be discussed in essence only. 1,6-8

According to this scheme, capillary stasis due to coronary occlusion or systemic hypotension produces varying degrees of starvation acidosis of the muscle cells, capillary hypercoagulability, sludging and thrombosis. The metabolic waste products accumulate and perpetuate this vicious cycle until the necrotic tissue reaction sets in after four to eight hours. Heparin and hypothermia are believed to have a viability-preserving effect by prevention of microblockade, whereas fibrinolytic agents would have a much more profound viability-restoring effect by dissolution of microblockade and relief of cell starvation.

Ischemic Hypercoagulability Following Coronary Occlusion: Evidence for ischemic hypercoagulability was obtained by thromboelastographic examination of blood draining from infarcted heart muscle. Non-wettable catheters were placed into the coronary sinus and a femoral artery of dogs with open chests which were subjected to temporary coronary occlusion. Serial coagulograms in twelve dogs, showed that sudden release of the coronary clamp produces a sharp drop of 30 to 70 per cent in the thromboelastographic clotting time. One typical experiment with coronary venous hypercoagulability after ninety minutes of temporary coronary occlusion is shown in Figure 4.

EFFECT OF FIBRINOLYTIC THERAPY ON EARLY INFARCTION

Evidence of ischemic microblockade in early infarction and its absence after fibrinolytic

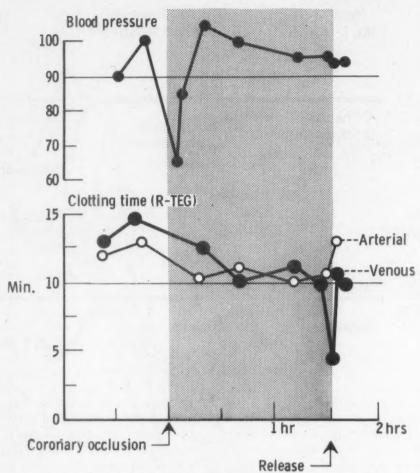


Fig. 4. Ischemic hypercoagulability of coronary venous blood after ninety minutes of temporary coronary occlusion. Serial determinations of thromboelastographic clotting time (i.e., clot reaction time: R) were performed on blood samples collected from catheters placed into the coronary sinus and a femoral artery. (Fresh non-wettable catheters were used for each sample.)

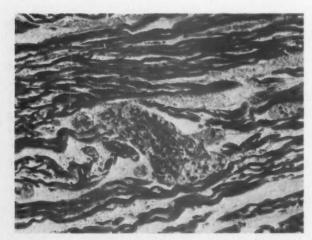


Fig. 5. Photomicrograph of a twelve-hour old untreated infarct showing the central zone with severe interstitial edema, shrinkage of muscle fibers, and massive platelet aggregation in a dilated vessel.

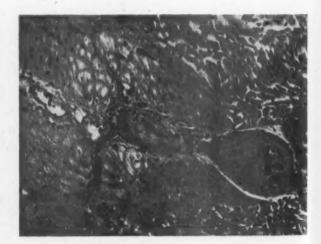


Fig. 6. Photomicrograph of the same infarct showing the marginal zone with thrombi in capillaries and venules.



Fig. 7. Photomicrograph of a thirteen-hour old infarct in which coronary circulation was restored after six hours of plasmin treatment. The central zone is shown with moderate interstitial edema and some focal necrosis of muscle cells with leukocytic invasion. Note the absence of thrombi, hemorrhage and vascular congestion.

therapy is presented in Figures 5 and 6. Figure 5 shows the central zone of a twelve-hour old untreated infarct with marked interstitial precipitation, edema and massive platelet aggregation in a dilated vessel. The marginal zone of the same infarct shows microthrombi in capillaries and venules (Fig. 6). The effect of fibrinolytic treatment is illustrated by the

photomicrograph of a thirteen-hour old infarct where capillary thrombi, vascular congestion and interstitial sludge are completely absent (Fig. 7). This change in the structure of the infarct occurred as early as one hour after the start of the plasmin treatment prior to lysis of the primary thrombus.¹

Correlation of Fibrinolytic Activity and Size of Infarct: To find out in principle whether this disappearance of microblockade represents a viability-restoring effect, we compared the size and structure of the infarcts in a treated and control group from one to twenty-eight days after three hours of temporary coronary occlusion.8 Figure 8 shows the correlation of varying degrees of fibrinolytic activity with infarct size and location of infarcts up to twentyeight days old. The non-lytic animals had confluent and large transmural infarcts in the majority of cases. Plasmin treatment given one hour before and for four hours after release of the clamp, despite constant dosage, produced varying degrees of fibrinolytic activity and associated changes in size, location and microstructure. The animals with marked fibrinolytic activity (lysis time of one hour or less) had 25 to 50 per cent smaller, often spotty, infarcts with regression into the apical and subendocardial regions.

Infarction	a	1	1 2	Da J 3	ys I 4	1 7	14	21	28
Large Confluent Transmural		000	0	000	0	00			
Confluent Apical and/or Subendocardial				0.0		0	0		0
Spotty Focal Intramural	•	•	•	:		0	•	0	25
Spotty Subendocardial			•••	•	Control O			0	
None	0				Fibrinolytic > 1 H Activity (1 H		1 H	•	

Fig. 8. Fibrinolytic salvage of heart muscle infarcted by three-hour temporary coronary occlusion. Correlation of fibrinolytic activity with evolution of infarcts (age, size, location). See text.

Conclusions

Salvage of ischemic non-necrotic myocardium may be effected as early as one hour after starting fibrinolytic therapy, long before lysis of the primary obstructing thrombus. This is explained by collateral penetration and restoration of microcirculation and cellular nutrition.

The rate of breakdown within an area of infarction is not uniform. The tissue vulnerability or the severity of cellular damage seems to be a function of duration and degree of ischemic anoxia, which depends on local variations of the collateral blood supply.

The location of clinical infarction without thrombosis in the same apical and subendocardial areas as residual infarcts after fibrinolytic treatment may not be a coincidence and may be due to regression of the infarct after spontaneous fibrinolysis in the natural history of infarction in man.

The profound effect of fibrinolysis upon acute tissue reactions should be born in mind whenever fibrinolytic agents are given for thrombolysis or testing purposes.

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DISCUSSION OF PAPER BY DRS. RUEGSEGGER, NYDICK, ABARQUEZ, REICHEL, CLIFFTON AND LADUE

DR. CLARENCE MERSKEY (Capetown, South Africa): Dr. Henrietta Lackner and I have made some studies of patients who had recently had acute myocardial infarctions. We studied the clot lysis time of these patients by the method of Fearnley and Lackner and also measured euglobulin lysis. The observations were made during the first fourteen days after the acute myocardial infarction. It was theoretically conceivable that the occurrence of the infarct had been associated with a depression of fibrinolytic activity. Fibrinolytic activity was variable after the acute episode. In many cases there was an increased tendency toward more rapid clot lysis in the days following the infarct. However, there were several patients who did not show this phenomenon and a few who even showed the reverse. We tried to correlate the changes in fibrinolytic activity with the plasma fibrinogen levels, the severity of the infarct and the treatment the patients had received. None of these correlations were positive except that we did notice that heparin appeared to accelerate both the euglobulin lysis time and the clot lysis time. This was tested in separate experiments in which other convalescent patients received 100 mg. of heparin intravenously. These and adequate control observations were continued for two hours after the injection. There appeared to be no doubt that heparin given intravenously in these doses was a potent accelerator of blood clot and euglobulin lysis in human beings.

DR. PAUL RUEGSEGGER (New York, New York):
There are numerous papers dealing with hypercoagulability or spontaneous lysis in ischemic heart
disease. We believe that our studies may provide
a link to show that there is a variation only in degree
of the same physiopathological disturbance between
angina pectoris with focal necrosis and confluent
infarction following coronary thrombosis and that
there may be a different physiopathology involved
in those infarcts which occur after surgical hypotension when the lytic system may be powerfully
stimulated and one does not find a clot if such a
patient should die. It seems advisable to give heparin
to all patients with heart attacks as soon as the first
symptoms occur before the microblockade has taken

Segmental Perfusion of the Coronary Arteries with Fibrinolysin in Man Following a Myocardial Infarction*

ROBERT J. BOUCEK, M.D. and WILLIAM P. MURPHY, JR., M.D., WITH THE TECHNICAL ASSISTANCE OF LEONARD S. SOMMER, M.D. AND IGNATIOS J. VOUDOUKIS, M.D.

Miami, Florida

RECENTLY the possibility of enzymatic digestion of a coronary thrombosis has captured the imagination of investigators. Attempts have been made in the past to supplement the blood supply of an ischemic myocardium by surgically opening the obstructed coronary segment, 1 transplanting a peripheral artery into the heart muscle, 2 or stimulating the pericardial capillaries to grow into the myocardium. These advances in the surgical and medical approaches to therapy are promising. However, the idea of enzymatic digestion of a coronary thrombosis spawns a host of new questions which require new answers.

The most fundamental of these questions is the actual pathogenesis of occlusive coronary arterial disease. The clinician implies that most coronary occlusions result from a thrombus. Hospital pathologists report the frequency of demonstrable coronary occlusion underlying a myocardial infarction as 40 to 80 per cent of all hearts examined. This wide range is the result of different technics for coronary examination. Cursory postmortem studies of the coronary arteries demonstrate occlusion in approximately 40 per cent, while the more elaborate injection methods show obstruction in 80 per cent. However, this is only one part of the needed information.

Of great importance is the character of the coronary occlusion. Himbert and Lenégre⁵ provide the clearest study of this. Nineteen of their 212 patients had no demonstrable occlusion. There were 517 occlusions equally divided be-

tween atherosclerosis and thrombosis. The thrombotic lesions predominated in the proximal portion of the anterior descending branch with an accompanying transmural infarction. In addition, and of the greatest importance to the considerations of this report, a recent thrombus was present only in 45 per cent of all thrombotic occlusions. The other 55 per cent were caused by old thrombi. Atherosclerotic occlusions produced transmural infarctions less frequently, occurring in approximately 30 per cent. Thus, even with an effective fibrinolytic agent properly delivered to the site of the thrombus, only 27 per cent of all patients with myocardial infarctions may be expected to benefit.

Another question is the time limit for development of irreversibility of myocardial injury. Savranoglu's data from a study of dogs indicate that irreversibility occurs within sixty minutes following transient coronary occlusion. These findings were in accord with the observation of submicroscopic changes occurring in the myocardium within sixty minutes of sustained hypoxia. It is apparent, then, that a patient with a coronary thrombosis may need to be treated effectively within hours.

The purpose of the present paper is to review methods of selection of patients for fibrinolysin therapy, the development of equipment for segmental perfusion of the coronary arteries, the precautions of administration of the clot-lysing solution, and the observations on the first eight patients treated by segmental perfusion of the coronary arteries with fibrinolysin.

^{*} From the University of Miami School of Medicine, Miami, Florida. This work was supported in part by the Developmental Fund of the Section of Cardiology of the University of Miami School of Medicine, by the Miami Heart Institute, and by Grant H-4794 of the National Institutes of Health, U. S. Public Health Service.

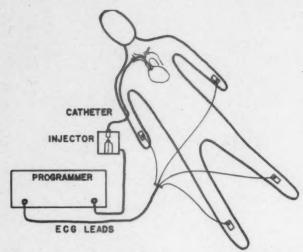


Fig. 1. Schematic diagram of cardiac programmed injection.

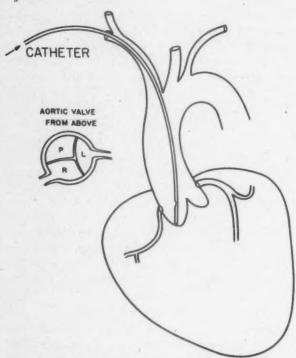


Fig. 2. Catheter placement for cardiac programmed injection.

METHODS OF APPROACH

Coronary Perfusion: The segmental perfusion of an artery may be accomplished by a major surgical procedure or, in the case of the coronary system, the vessels may be perfused through a catheter passed to the root of the aorta.

It is necessary to deliver this perfusate during the period of coronary filling. In order to effect proper timing, the electrocardiogram is utilized as an activating impulse. An electronic cardiac programmer activated by the R wave has been used in our laboratories for certain studies in cardiovascular physiology.8 The impulse starts a small pump which

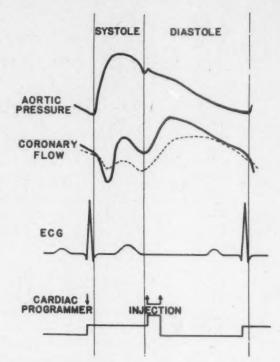


Fig. 3. Programmed injection synchronous with period of maximum coronary blood flow.

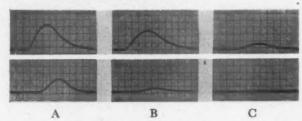


Fig. 4. Myograms showing the effect of fibrinolysin (thrombolysin in tyrode solution without glucose) on myocardial contraction of seventy-two hour chick embryo heart, at temperature of 28.5 °c. Paper speed: 50 mm./sec. Time of exposure to fibrinolysin three minutes (upper) and fifteen minutes (lower). A, 6 units/ml. B, 60 units/ml. C, 600 units/ml.

injects the desired volume of solution through a cardiac catheter. The tip of the catheter is impinged in the sinus of Valsalva so that the coronary ostium is in juxtaposition with the catheter orifice (Figs. 1 and 2).

Early work with dogs established the critical period of coronary flow to be in the beginning of the middle third of diastole (Fig. 3). The timing was established by following the passage of radiopaque material into the coronary system of dogs. If injection is made in other phases of diastole, inadequate perfusion of the coronary arteries results.

Character of Segmental Arterial Perfusion: The lysis of a clot in a tubular system such as an artery is best accomplished by delivering the proteolytic enzyme in intermittent injections near the site of the clot. This fact was established by comparing the resolution of thromboplastin-induced clots in narrow plastic

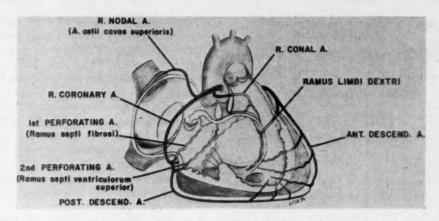


Fig. 5. Right anterior oblique view of coronary blood supply to the atrioventricular node and the right bundle branch.

tubes by first layering the clot with proteolytic enzyme and then exposing a similar sized clot to brief periods of intermittent injection. The results showed that while layering a clot with fibrinolytic solutions for a period of twenty-four hours dissolved only one-fourth of the clot, three or five minutes of intermittent flushing dissolved three-fourths. This theory of the superiority of intermittent flushing was tested on the saphenous system of the dog. A thromboplastin-induced thrombus was lysed within five minutes by the intermittent injection of 0.1 ml. of fibrinolysin (Actase,* 1,000 units per ml.). Parenteral administration of fibrinolysin, on the other hand, resulted in only partial resolution of the clot even after six to eight hours.

The Concentration of Fibrinolysin: Fibrinolysin† has a toxic effect upon the myocardium of the heart of the chick embryo (Fig. 4), and in concentrations of or exceeding 60 units per ml., contractions ceased.

Prolonged administration of large doses of fibrinolysin sufficient to depress circulating fibrinogen during the initial forty-eight to seventy-two hours appeared undesirable since the process of fibrinogenesis depends to a great extent upon the existence of a fibrin clot. If the deposited fibrin in the injured myocardium becomes depolymerized, then healing will be impaired.

Problems of Evaluation of Response: Segmental perfusion of the coronary artery with an effective fibrinolytic solution will resolve a fresh thrombus. This may be visualized by proper coronary arteriography. However, the existing myocardial irritability due to ischemia may be aggravated by the presence of the radiopaque materials. Consequently, coronary arteriography was not used at this

Standard means of evaluating patients by their

* Actase is the Ortho Pharmaceutical Corporation preparation of fibrinolysin.

† Fibrinolysin (as Thrombolysin) was kindly supplied by Merck Sharp & Dohme, used in strength of 1,000 units per ml. clinical response, the electrocardiogram and laboratory tests such as serum enzyme determinations were used. It is expected that tissue enzymes would be liberated and electrocardiographic changes would occur even with the re-establishment of the patency of the coronary artery because of irreversibly damaged muscle as the result of prolonged ischemia.

METHODS OF PROCEDURE

Case selection was based upon the necropsy findings of Himbert and Lenègre.⁵ Principally, young persons without previous history of myocardial infarction but with an occlusion of the ariteron descending branch or the right coronary artery were treated.

The knowledge of the location of the blood supply to different parts of the conduction system is helpful in determining the site of occlusion (Fig. 5). This location may be predicted with considerable accuracy by careful examination of the electrocardiogram. For example, the electrocardiogram determined that the occlusion of the right coronary artery in one patient (Case 1) was located proximal to the ramus septi fibrosi because of the associated atrioventricular conduction disturbance with the posterior wall infarction. One patient (Case 3), on the other hand, had involvement of the right coronary artery distal to the ramus septi fibrosi.

Sedation: The patients were kept comfortable with the necessary amounts of morphine. Pentobarbital was also used for sedation during the procedure.

Catheterization of the coronary sinus was made by the passage of a No. 7 modified Lehman catheter through the right brachial artery. Positioning of the catheter was accomplished by fluoroscopic guidance and checked by a spot film before the procedure started. It was not always certain which vessel was involved, particularly when the injury potential was marked or when right bundle branch block was present. For this reason, perfusion of the right coronary artery would be made with one-third of the solution, and

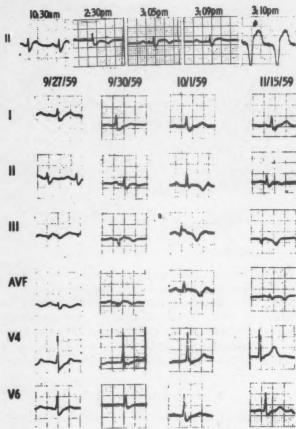


Fig. 6. Case 1. Electrocardiogram during coronary perfusion, which started at 2:30 p.m. on September 26, 1959, and the follow-up tracings. Note the progressive atrioventricular delay during the perfusion and the subsequent 2:1 block, particularly on October 1, 1959.

then by repositioning the catheter into the posterior non-coronary sinus, both arteries would be perfused with the remaining two-thirds.

The use of the right brachial artery facilitated the passage of the catheter into the root of the aorta. In two cases the radial pulse was depressed producing mild paresthesia and weakness of the hand.

Administration of Fibrinolysin: Injection of fibrinolysin was made in the diastolic period by the use of the programmed injection. Experience with one patient (Case 1) indicated that injecting with each cardiac cycle was dangerous. Consequently, 0.3 ml. of the perfusate (made up of 1,000 units of fibrinolysin per ml. in normal saline solution) was delivered with every fourth cardiac cycle. Each milliliter was supplemented with 0.4 mg. of heparin because of the reported enhancement of lysis by this combination. It was important to perfuse the arteries within the first few hours.

After each 25,000 units of fibrinolysis the patient was reappraised by physical examination and electrocardiogram and the position of the catheter checked by fluoroscopy. A maximum of 75,000 units of fibrinolysis was delivered into the root of the aorta in approximately one hour.

Postperfusion Therapy: A slow intravenous drip of 5 per cent glucose in water was continued for twelve hours, delivering 125,000 units of fibrinolysin and 50 mg. of heparin. Parenteral heparin and oral coumarin were administered after the first twenty-four hours. After the initial forty-eight hours only the coumarin preparation was continued. The twelve-hour maximum dose of 200,000 units of fibrinolysin (Thrombolysin) was adhered to upon the recommendation of the manufacturers. This apparently is an effective systemic dose and the high concentration delivered to the coronary arteries approximated the calculated dose which the myocardium could tolerate.

Laboratory Studies: A complete blood count, prothrombin time, Lee-White clotting time, serum glutamic oxaloacetic transaminase, sedimentation rate, electrocardiogram, urinalysis and roentgenograms of the chest were taken. Special blood studies, including serum fibrinogen, were performed in the laboratories of the Miami Heart Institute and will be reported elsewhere.

Postperfusion Studies: The electrocardiogram was taken and serum glutamic oxaloacetic transaminase determined twice per day for the first twenty-four hours and then daily for the following seven days.

RESULTS

CLINICAL OBSERVATIONS

During the Perfusion: All patients tolerated the procedure without incident' with the exception of the first patient (Case 1). In this patient, injection was made with each cardiac cycle and, during the procedure, blood pressure fell despite favorable changes in the electrocardiogram. At first, pulse was steady and slow. Then, suddenly, an idioventricular rhythm developed. At this point the procedure was discontinued and emergency measures for the ensuing shock-like state were instituted (Fig. 6). Fortunately, a prompt response followed and the patient had an uneventful convalescence. This was the only complication of all coronary perfusions.

In one patient (Case 5), the persistent precordial distress disappeared during the perfusion. In another patient (Case 3), moderate hypotension attending the infarction returned to normal during the perfusion. Evidence of epicardial injury in the same patient disappeared within seven minutes during the first perfusion (25,000 units). Pulse rate, blood pressure and respiration rate were not consistently altered in any of the patients. Two patients suffered from nausea at the end of the procedure, and in one this was accompanied by vomiting.

During the Convalescent Period: All patients

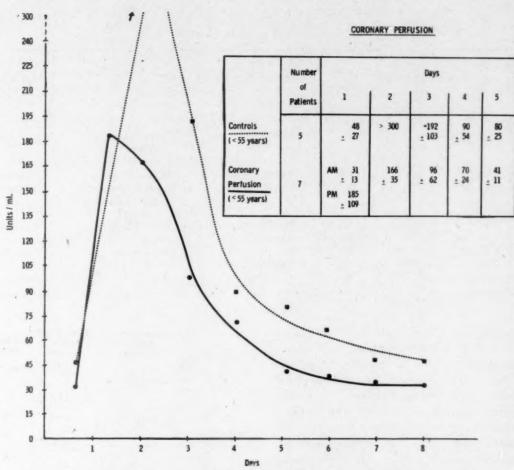


Fig. 7. Course of serum transaminase response in patients with myocardial infarction (dotted line) compared with serum enzyme rise in patients treated by coronary perfusion of fibrinolysin (solid line). (Only the first seven patients of Table 1 are included.)

had an unusually mild course in the hospital. A mild pyrexia occurred in most patients which persisted for twenty-four to forty-eight hours. After this time the course was without incident except for one patient (Case 6) who had a distressing pericarditis for eight days. Diagnosis made from changes in the electrocardiogram revealed pericarditis in three patients.

Serum Glutamic Oxaloacetic Transaminase Response: The enzyme activity increased rapidly in the serum of all patients, reaching its maximum within twenty-four hours. Afterwards it declined exponentially, approaching the asymptote by the sixth day. When this was compared with serial serum enzyme determinations from five other patients of comparable age not perfused (Fig. 7), a significantly lower curve was revealed in the treated patients. Furthermore, in the non-perfused patients the markedly elevated serum enzyme concentration remained at a high level for an additional twenty-four hours

and the enzyme concentration remaining after five days was greater.

In all patients coronary perfusion was started within four to twelve hours after the onset of chest distress (Table 1). In one patient (Case 2), the serum enzyme concentration did not rise to extreme concentrations (157 units); however, the return to normal levels was not as rapid as in the other patients. A patient (Case 4) whose infarction was nine hours old had an elevation of serium transminase of 216 units which persisted at a high concentration for three days. Serum enzyme elevation was the mildest (113 and 82) in two patients (Cases 7 and 8) who were perfused in less than five hours following the onset of precordial pain.

ELECTROCARDIOGRAPHIC STUDIES

Electrocardiogram During Perfusion: Caution is required in interpreting early electrocardiograms, for the changes in the record are ex-

TABLE 1 Clinical Data in Eight Patients

Case No.	Age (yr.) Sex	Age of Infarct by History (hr.)	Angina	Blood Pressure (mm. Hg)	Complications	Location of Infarction
1	50, M	6–7	No	120/90	Intermittent 2nd degree A-V block	Posterior
2	42, F	7-12	Yes	135/90 (stable)	Pericarditis	Anterolatera
3	52, M	71/2	No	110/70 (stable)	Intermittent right bundle branch block	Posterior
4	38, M	9	No	105/75 (stable)	Pericarditis	Posterior
5	49, M	5	No	100/60 (stable)	None	Lateral
6	35, M	5	No	130/90 (labile)	Pericarditis	Anteroseptal
7	41, M	4-5	No	170/100 (labile)	None	Posterior
8	43, M	4	No	110/70	None	Posterior

tremely capricious. Electrocardiograms during this early period indicate that marked injury potentials characterized by the usual displacement of the J wave and the deformation of the S-T segments may disappear entirely during the initial three or four hours without any therapy. This instability did not occur in all patients but no consistent guides were present to separate the labile from the stable pattern. For this reason the electrocardiogram was of value principally in locating the area of involvement and in interpreting any complicating conduction disturbances

Continuous electrocardiographic monitoring was essential as illustrated by Case 1 in which, during the course of perfusion (Fig. 6), an ectopic ventricular rhythm developed and prompt remedial measures were taken.

While the perfusion was underway in one patient (Case 3), the marked J wave displacement and ST segment alteration disappeared during the initial seven minutes (Fig. 8). The record in Case 8 approached normal following perfusion (Fig. 9). In the other five patients, insignificant changes in the record occurred during or following the perfusion.

Electrocardiogram after Perfusion: An interesting phenomenon occurred in two patients (Cases 5 and 8). A normal electrocardiogram was noted within six hours following the perfusion only to have the usual T wave changes of an evolving myocardial infarction appear on the subsequent daily records (Fig. 9). In the other patients, the electrocardiographic changes present at the time of perfusion proceeded in the predictable

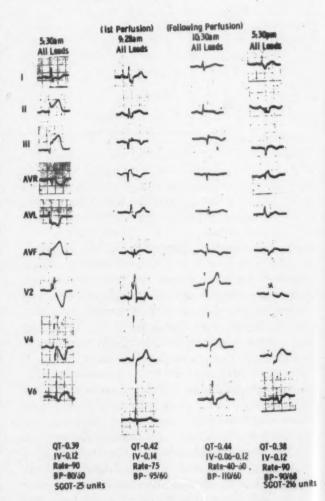


Fig. 8. Case 3. December 24, 1959. Right coronary perfusion. Rapid evolution of acute injury pattern following perfusion. Note intermittent right bundle branch block complicating the myocardial infarction of the posterior wall.

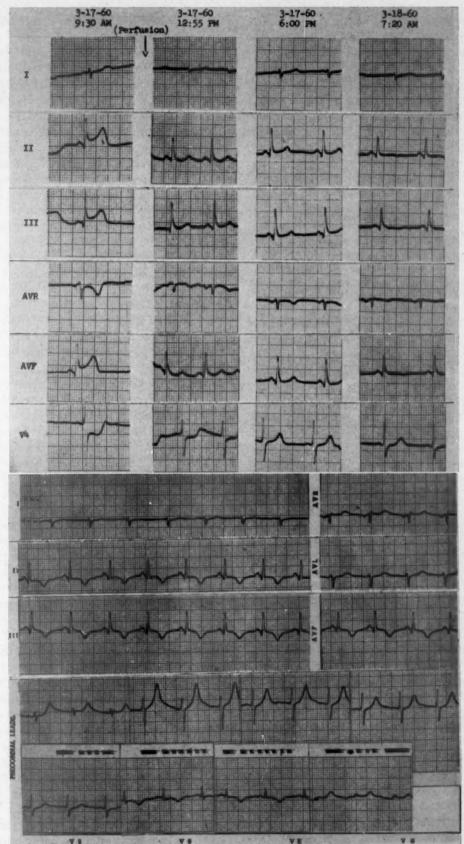


Fig. 9. Case 8. Coronary perfusion. Rapid evolution of the myocardial infarction of the posterior wall. Note normal electrocardiogram at 6:00 p.m. on March 17, 1960, and the subsequent T wave evolutionary changes seen on tracing on March 19, 1960.

manner of an infarction. There was one consistent observation, however. Only in the patients with a complicating pericarditis did an S-T segment elevation persist after the perfusion.

Electrocardiographic Follow Up: A six-month follow-up record in one patient (Case 1) and a five-month postinfarct record in another (Case 2) reveal evidence of the receding infarct. In the first patient (Case 1) there developed a larger Q wave in leads III and aVF, while in the second (Case 2) R wave was absent in leads V₁ to V₄. A four-month follow-up period in another patient (Case 3) revealed the development of Q waves associated with a myocardial infarction of the posterior wall with the T wave changes returning toward normal.

CLINICAL OBSERVATIONS AFTER DISCHARGE FROM HOSPITAL

Two patients (Cases 1 and 3) have returned to work. The first is employed as a salesman traveling forty to fifty miles per day and walking almost three miles per day without symptoms; the second patient is working two to three hours per day as a clerk and is without symptoms. One patient (Case 2) has persistent angina and is not able to do anything other than minor work around the house. Inadequate time has elapsed to properly evaluate the other five patients.

COMMENTS

The segmental perfusion of the coronary arteries is accomplished without complications. This was found to be true for the eight cases reported herein. Administering the proteolytic enzyme directly into the artery has such obvious advantages as a great local concentration without comparable elevation of the systemic concentration. In addition, the solution may be administered intermittently, a procedure which appears to enhance proteolysis.

Evaluating the effect of segmental arterial perfusion is not simple. A number of observations from this study are suggestive of a beneficial result. Foremost in the means of evaluation are the serial determinations of serum glutamic oxaloacetic transminase. The height of the enzyme rise during the initial forty-eight hours was significantly less in the perfused patients. LaDue et al. 10 and Mason and Wroblewski 11 have reported a correlation in the degree of the serum enzyme rise and the extent of infarcted myocardium. The data presented in the present report may then be interpreted to suggest the preserva-

tion of a larger amount of viable muscle in the perfused patients.

A remote explanation of the depression of enzyme rise in the perfused patients might be the direct proteolysis of the liberated tissue enzyme by the infused fibrinolysin. Plotting the decline of enzyme activity after the forty-eighthour period on semi-log paper revealed a linear regression from which a half-life determination was calculated. The half-life of the elevated serum enzyme in the treated patients was 1.8 days. The peak of the enzyme rise for the control patients was unknown; however, according to the available data the calculated half-life was similar to that of the treated patients. Although in both cases the declining slopes were parallel, that of the treated patients consistently showed lesser amounts of enzyme released. This finding is additional support for the concept of muscle preservation by the coronary perfusion technic.

The rise of serum transaminase despite the perfusion indicates that irreversibility of myocardial injury occurs within four hours. The electrocardiograms in two patients (Cases 2 and 4) indicated no change as the result of the perfusion and their serum enzyme elevation was slow in returning to the normal level. It should be noted, however, that one patient (Case 2) had a history of a bilateral oophorectomy twelve years prior to admission and recurring angina during the preceding twelve months. The work of Roberts et al.12 would suggest a diffuse coronary arterial disease as the basis of her symptoms and that the acute occlusion probably was atherosclerotic in nature. The infarction in one patient (Case 4) existed for nine hours prior to the perfusion. No change was observed in this patient's electrocardiogram during the procedure and the elevated serum enzyme remained longer than in any of the other patients. It is possible that the underlying mechanism of this occlusion was also atherosclerosis.

Further evidence for the possible beneficial effects of segmental coronary perfusion was the disappearance of the acute injury phenomenon noted particularly in one patient (Case 3) and, in addition, the absence of S-T segment elevation in any of the patients (except those with pericarditis) during the convalescent period. Yet, T wave aberrances evolved in a classic pattern. The elaboration of tissue enzyme in all the patients and the evolutionary T wave changes indicate that irreversible muscle damage had occurred. However, this damage may have been limited by the coronary perfusion.

The depression of myocardial contraction by fibrinolysin was surprising. A suspicion that this might occur came after perfusing the patient (Case 1). It will be recalled that the first electrocardiogram was reverting toward normal while paradoxically the blood pressure was falling. Testing the fibrinolysin against the heart of the chick embryo caused a depression of the strength of contraction and a lengthening of the relaxation period. The simultaneously determined electrocardiograms were unchanged despite the completely suppressed muscular contraction. No known mechanism for depression of the muscular contraction by fibrinolysin is apparent. The enzyme did not influence the membrane phenomenon which produced the electrocardiogram. However, because of this myotonic action, a definite concentration maximum exists. For this reason, fibrinolysin was injected with every fourth cardiac cycle, a technic which obviated further difficulties.

The key question is whether a thrombus underlay the myocardial infarctions in the eight patients studied and whether the thrombus was depolymerized or digested by the perfusate. The indirect evidence which was presented suggests that this occurred in probably six of the eight patients. However, great reservations must be retained until the methodology of study of the patency of the coronary artery improves and an adequate follow-up study of patients can be made.

A great deal of work and experience is required before the true value of fibrinolysin therapy is known. A more careful selection of patients suspected of having an acute occlusion due to a fresh thrombus may permit greater success, paricularly if the lytic agent is used within the first few hours after the infarction. In any event, segmental arterial infusion appears to offer distinct advantages over any other route of administration.

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Experiences with Clot-Lysing Agents in Coronary Thrombosis*

ISAAC H. RICHTER, M.D., F.A.C.C., FRANK MUSACCHIO, M.D., EUGENE E. CLIFFTON, M.D., AHMAD NASSAR, M.D., LOUIS KAPLAN, M.D., ARMANDO G. FAVAZZA, M.D. and ALI AKBARI, M.D.

Brooklyn, New York

ANTICOAGULANT therapy in coronary thrombosis is designed to prevent further thrombus formation and prevent spread of infarction. The aim of thrombolysin therapy is to restore patency to the vessel and thus permit restoration of function to viable tissue.

Ruegsegger et al.1 have studied the effect of plasmin administration upon experimentally produced coronary thrombi. In a group of eight dogs undergoing treatment, complete lysis of coronary thrombi was achieved in four animals in three to seven hours and in the other four partial lysis was achieved. They also found a marked decrease in capillary congestion, less edema and complete absence of capillary thrombi in the entire group undergoing treatment, in contrast to eight untreated control dogs. In the latter, the thrombi were stable for as long as fifteen hours after they were produced. DeLeon et al.2 have studied the value of plasmin administration in eight patients with acute myocardial infarction. Their results showed striking relief of angina in six patients and electrocardiographic improvement in three.

MATERIAL AND METHODS

In view of these encouraging data, we administered thrombolysin to sixty patients with various thromboembolic diseases, forty-five of whom had myocardial infarction. Of these forty-five there were thirty men and fifteen women. Eighteen of the men had infarction of the posterior wall and twelve had infarction of the anterior wall. For the women patients, there were seven with anterior infarction and eight with posterior infarction.

The age distribution, associated diseases and time from onset of the coronary occlusion to the beginning of treatment are shown in Tables I, II and III. Table I shows that there were thirteen men and one woman in

TABLE I Age Distribution

Age (yr.)	Men	Women
30 to 39	3	0
40 to 49	7	1
50 to 59	3	1
60 to 69	10	. 8
70 to 79	7	6

the age group thirty to fifty-nine years. In the sixty-to sixty-nine-year age group, there were ten men and eight women and in the seventy- to seventy-nine-year age group there were seven men and six women. Twelve patients had no previous associated diseases and thirty-three had one or more previous associated diseases (Table π).

The patients have been divided into groups as to the time thrombolysin therapy was started from the

TABLE II
Associated Diseases

Disease	Men	Women
Hypertension	6	. 8
Diabetes	4	6
Previous myocardial infarc- tion	5	1
Previous angina	9	5
Peptic ulcer	1 .	0
Previous congestive heart failure	2	5
Phlebitis	0	1
Uremia and anemia*	1	0

* Laboratory report obtained following the onset of therapy.

^{*} From the Vascular Service, Department of Medicine, Coney Island Hospital, and the Coney Island Hospital Research Institute, Brooklyn, New York. This study was supported by Grants H5055 and H2867 of the National Institutes of Health through the Sloan-Kettering Institute for Cancer Research.

TABLE III
Time from Onset of Myocardial Infarction to Beginning
of Thrombolysin Therapy

Hours	Men	Women
3 to 5	8	3
6 to 10	12	6
11 to 20	6	3
21 to 50	3	
56	0	1
72	0	1
120	1	1

time of onset of symptoms (Table III). The majority received therapy within ten hours of the onset of the attack.

Fibrinolysin Administration: All patients received fibrinolysin* by continuous intravenous drop. The dosage was approximately 1,000 per kg. body weight per hour for six to eight hours per day for four days. The diluent was 5 per cent dextrose in water, 150 cc. for each two-hour period. Prothrombin times and whole blood clot lysis times were determined every four hours during therapy. Electrocardiograms, transaminase and sedimentation rate determinations were recorded daily. Blood counts and urinalysis were performed every other day. Benadryl® was given before and during therapy. Anticoagulant adminis-

* Thrombolysin, Merck Sharp & Dohme, West Point, Pennsylvania.

tration was started a few hours after the last dose of thrombolysin.

RESULTS

Of this group, thirty-nine patients survived and six died (three women and three men). Two of the six patients died two and a half and four hours after admission before an adequate dose of thrombolysin could be administered. All six patients were in shock and/or cardiac decompensation at the time of admission (Table IV).

Thirty-eight patients had permanent and striking relief of precordial pain within a short time after thrombolysin administration was begun and during their hospital stay. Seven patients (four men and three women) experienced intermittent precordial pain during and after therapy. One woman experienced precordial pain two days after thrombolysin therapy and died.

During the patients' stay in the hospital, there were no significant regressive changes in the electrocardiograms, except in one patient in whom the Q waves in lead V₄ disappeared.

Four patients with hypertension had a drop in systolic blood pressure ranging from 40 to 70 mm. Hg during therapy without any apparent ill effects.

TABLE IV
Characteristic Features of the Patients Who Died

Patient	Age (yr.) and Sex	Onset until Therapy (hr.)	Enzyme Received (1,000 units)	Associated Diseases	Site of Infarct	Reaction During Therapy	Time Between Onset of Therapy and Death	Condition of Patient Prior to Therapy	Cause of Death
R. B.	63, F	4	150	Hypertension; previous myo- cardial infarc- tion	Posterior	None	2.5 hr.	Shork	Shock
P. B.	80, F	16	1,600	Diabetes; hyper- tension; angina; congestive heart failure	Posterior	None	5 days	Shock; diabetic acidosis	Possible re- current coronary occlusion
F. M.	73, M	. 8	280	Hypertension	Posterior	None	4 hr.	Congestive heart failure; shock	Congestive heart failure
J. C.	60, F	72	1,200	Diabetes; hyper- tension	Anterior	None	2 wks.	Shock; conges- tive heart fail- ure; blood urea nitrogen 49 mg.	Congestive heart failure
C. V.	67, M	18	875	Angina; conges- tive heart fail- ure	Anterior	None	14 hr.	Shock	Shock; conges- tive hear failure
М. Р.	77, M	12	1,000	Angina; previous myocardial in- farction; con- gestive heart failure; uremia; anemia	Posterior	Rectal bleed- ing	6 days	Congestive heart failure; cyanosis, disoriented	Congestive heart failure; uremia

Table v Side Reactions During Therapy

Reaction	No. of Patients
Vomiting	3
Abdominal pain	1
Superficial ecchymosis	1
Flushing	1
Bleeding from open carbuncle	1
Hematemesis (history of peptic ulcer)	1
Hematuria	1
Rectal bleeding*	1

* This patient died six days later in uremia and congestive heart failure.

TABLE VI Rectal Temperatures Before and During Therapy

Temperature (degrees Fahrenheit)	No. of Patients
Before	Therapy
97	4
98	10
99	25
100	5
102	1
During '	Therapy*
99	3
100	9
101	19
102	11
103	
104	1

Note: Fraction of degree of temperature was eliminated for simplicity.

* No temperature recorded in two patients who died shortly after starting thrombolysin therapy.

Side Effects: The side reactions and changes in temperature caused by the fibrinolysin infusions are shown in Tables v and vi.

The whole blood clot lysis was complete in two patients in two hours with respective prothrombin times of thirty and twenty-four seconds. One patient had complete lysis in twelve hours and another in sixteen hours with respective prothrombin times of 18.4 and 17.2

TABLE VII Prothrombin Times During Therapy

Prothrombin Time (sec.)	No. of Patients
18 to 20	17
21 to 25	10
26 to 30	5
60	2

seconds. Table vII lists the changes in prothrombin times during therapy.

COMMENTS

Coronary artery occlusion with infarction presents a difficult therapeutic problem. Several factors are implicated. The results of coronary occlusion depend on the size and location of the vessel, the rate of formation of the thrombus, the age of the patient and the state of the general circulation. There are other variables worthy of note: (1) location and severity of pain to cause patients to seek early medical aid; (2) time of onset of pain in relation to the occlusion; (3) the time thrombolysin therapy is started; (4) the presence of myocardial insufficiency and shock; and (5) the availability of more effective drugs in the treatment of shock in myocardial infarction.

Tennant,⁸ Prinzmetal⁴ and others have demonstrated that by ligating a major branch of a dog's coronary artery, the myocardium becomes cyanotic in fifteen seconds and stops contracting in one minute. During the first fifteen minutes these changes are reversible and after twenty to twenty-five minutes are irreversible. In the next few hours the gross and microscopic changes, characteristic of an infarct, develop.^{5,6}

Ruegsegger's work in experimentally produced infarcts in dogs demonstrated that total lysis of coronary thrombi occurred in three to seven hours in four of eight dogs receiving thrombolysin therapy. In the current series, eleven patients received thrombolysin therapy from three to five hours after the onset of appreciable degree of pain; eighteen patients received it from six to ten hours and the rest from eleven to 120 hours.

Serial electrocardiographic changes were reported. In all cases the changes were considered to be consistent with the diagnosis of acute myocardial infarction. The electrocardiographic evolution of the infarction did not deviate from the usual course. In one patient who received

thrombolysin six hours after the onset of pain the Q wave in lead V₄ disappeared.

Recurrence of pain during therapy occurred in only seven patients. One of these patients died following an attack of precordial pain, presumably due to recurrent coronary occlusion two days after thrombolysin therapy. She was not adequately treated with Dicumarol. The remainder of the patients had a prompt, permanent cessation of pain after thrombolysin was started. It is significant to note that embolization did not occur in any patient in this series.

In our studies, we found that during and after therapy all patients, except for twelve, had a significant rise in temperature. It cannot be determined how much of the rise was due to the myocardial infarction or its complications, the thrombolysin therapy or a combination of the two. There were ten patients with other side reactions. Six of these had minor reactions and four had significant evidence of bleeding. One showed rectal bleeding and evidence of uremia and anemia and died six days after admission. The cause of death was attributed to congestive heart failure and uremia.

SUMMARY

Thrombolysin was administered to forty-five patients with acute myocardial infarction. Anticoagulants were not administered during thrombolysin therapy but were begun in most patients a few hours after administration of thrombolysin was stopped. There were thirty male
and fifteen female patients.

Of this group, six died and thirty-nine survived. Those who died were admitted to the hospital either in shock and/or congestive heart failure. Significant side reactions were noted in four patients.

In view of the results in experimental animals and the results obtained in our series, early institution of thrombolysin therapy is imperative for optimum results.

This is a preliminary report. The patients who survived are being re-evaluated and comparisons are being made with a control group on a long term basis in the follow-up clinic.

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DISCUSSION OF PAPERS BY DRS. BOUCEK AND MURPHY; AND RICHTER, MUSACCHIO, CLIFFTON, NASSAR, KAPLAN, FAVAZZA AND AKBARI

DR. JOHN WILSON (Hartford, Connecticut): It is apparent now that an adequate drug is available which certainly seems to be safe but we need more control data. Double blind studies will be necessary before we know what effect this drug is causing in coronary thrombosis.

DR. IRWIN NYDICK (New York, New York): I would first like to amplify Dr. Ruegsegger's previous paper, if I may. I think Dr. Ruegsegger pointed out that this is really a two phase problem. First, what chance is there of lysing a major coronary thrombus? Second, what are the possibilities that beneficial effects will be produced in the myocardium? Certainly if the myocardium is totally and irreparably damaged in this short period of time, the lysis of a fresh coronary thrombus is merely an academic exercise. For this reason, we first demonstrated the feasibility of the lysis of large coronary clots in the dog, and then demonstrated in long term experiments that the myocardium would be partially spared. If therapy was started two hours after occlusion of the coronary artery by a clamp and then the clamp removed at the third hour this reduced the size of the resulting myocardial infarcts.

Dr. Boucek has presented experiments demonstrating the difficulties encountered in evaluating the first phase of this problem; namely, the influence on the coronary clot itself. As he noted, Dr. Lenégre and others have shown that probably no more than 40 per cent of patients at autopsy demonstrate a fresh coronary thrombus as a target for therapy. I regret that Dr. Boucek does not have coronary arteriograms demonstrating whether or not these vessels were reopened; that would even be of greater importance in aiding our evaluation of the success of this therapy.

Turning our attention from the coronary thrombus to the myocardium itself, what hope do we have of treating enough patients during the early reversible phase of myocardial infarction to warrant such therapy? I think that the studies are warranted and we are looking forward to a large control series because both Dr. Boucek and Dr. Richter will agree that the number of patients in their groups do not warrant statistical analysis at this time. If we consider the joint study of Drs. Wright, Marple and Beck, a well controlled group of 1,031 patients was studied, and their data are under attack from many sources as to its validity. If we now think of comparing a thrombolysin-treated group of patients with an anticoagulated group of patients (certainly few of us would use untreated patients as a control group), the problem in the statistical evaluation of these data is brought into sharp focus.

Finally, what autopsy percentage will there be? For example, if we see 100 patients with myocardial infarction, the average mortality in general hospitals is about 25 per cent excluding the patients who die before they are admitted to the hospital. An excellent autopsy rate would be 40 per cent, so that of every 100 patients treated we have the opportunity of careful study of the coronary artery system and myocardium of perhaps ten patients. Therefore, although the fibrinolytic therapy of myocardial infarction opens a broad new field, we should accept any preliminary results with many reservations, but we all hope that the final results follow the encouraging trend of these

early reports.

DR. BENJAMIN MANCHESTER (Washington, D. C.): I have been interested in the treatment and prevention of thromboembolic disease for the past seventeen years, using available anticoagulants. Maintaining patency of the coronary arteries with anticoagulants is sometimes frustrating and reflects the limitations of the currently available regimen. It is often disconcerting to discover the presence of an acute thrombophlebitis in a patient receiving oral anticoagulants. The rationale, however erudite, is unacceptable to the patient, as well as the house staff and attending physicians. What is the explanation for the development of a thrombus in a vein in a patient receiving anticoagulants for the purpose of preventing such a complication?

The answer is that oral anticoagulants do not possess the wide spectrum of activity to inhibit all the factors participating in clot formation. In spite of their limitations, the literature is replete with abundant proof of the role of oral anticoagulants in reducing mortality and in preventing thromboembolic disorders.

Fibrinolysis provides another modality for restoring patency of a blood vessel recently occluded by a thrombus. Fibrinolysin administration alone will not maintain a vessel patent unless oral anticoagulants are employed at the same time and their administration is continued for several weeks after the patient stops receiving fibrinolysin. A hypercoagulable state develops when fibrinolysin is withdrawn. Without oral anticagulants, thromboembolic complications may follow.

At the present time, fourteen patients with acute coronary occlusion have received fibrinolysin and oral anticoagulants, and an equal number of control patients have received only oral anticoagulants. There were four deaths in each group. The remarkable observation has been in the electrocardiograms of the fourteen patients receiving thrombolysin. Q wave changes were reversed in the first 120 hours in seven instances. The concept that a Q wave represents tissue death is a fallacy. Rather, it is evidence of loss of electrical activity of the injured heart muscle. It is my first experience to observe such rapid disappearance of the Q wave during treatment of acute myocardial infarction.

I am pleased that Dr. Richter found that the Q wave disappeared in one of his patients. Dr. Boucek, in his presentation, demonstrated striking changes in S-T and T waves, which can be characterized as the evolution of myocardial infarction in the process of healing. I believe he observed other changes, especially in intraventricular conduction and a reduction in the size of the Q wave in several of the electrocardiograms. Although Dr. Boucek did net comment on

them, I think they are noteworthy.

One observation made by Dr. Boucek requires further emphasis. If it is true that 50 per cent of the patients who die of myocardial infarction fail to show any evidence of coronary thrombosis at necropsy, the value of fibrinolysin therapy may be difficult to assess. If fibrinolysin is administered, and at autopsy a thrombus is not demonstrable in the coronary tree, it would be reasoned that its absence was due to fibrinolysis. It would follow that it is equally important that we keep our minds patent, as well as our vessels, if we aim to assess the therapeutic efficiency of fibrinolysis.

DR. MASON GUEST (Galveston, Texas): Dr. Boucek, have you checked whether or not the preparation used has a depressing action on the myocardium? This depressing action might be due to a contaminant and not to fibrinolysin. I ask this question because in some early work in Dr. Seegers' laboratory on fibrinolysin preparations, we found the preparations studied were contaminated with a factor which we called vascularin. This polypeptide produced hypotension, depression of the myocardium and other changes. It would be important to know whether or not current preparations also contain this factor.

From the Floor: I have been under the impression that the abnormal Q wave in the electrocardiogram in myocardial infarction represents death of tissue. Does this mean that dead tissue has been

revived?

DR. JOHN S. LADUE (New York, New York): Dr. Boucek, do you think that in view of some of the previous remarks that have been made at this conference the giving of activator might be more reasonable than the giving of plasmin into the coronary arteries?

DR. ROBERT J. BOUCEK (Miami, Florida): The question concerning coronary arteriography is an obvious

one. Coronary angiograms should have been recorded before and after the segmental coronary perfusion. However, the risk of this procedure at a time when there is a membrane instability due to the hypoxia, has caused us to postpone this procedure pending further laboratory work. We did not believe our experience justified its use in human patients. Our laboratories are exploring the possibility of the use of I¹³¹-labeled albumin injected into the coronary tree before and after the lytic procedure with the hope that a concentration difference may be noted by proper scanning procedures.

Dr: Guest asked about a contaminating myocardial depressant in the fibrinolytic solution that we have used. This was based on his previous observations. We have observed the myocardial depression in our first patient and subsequently in laboratory work with the heart of the chick embryo. This depressant acts on the contractile protein, as the electrocardiogram of the embryonic heart tissue was not affected by the

fibrinolytic solution.

Regarding the combined use of fibrinolysin with an activator such as streptokinase, the preparation which was used in these studies was a combination of the fibrinolysin plus the activator. As indicated, we added a small amount of heparin hoping to enhance the clot lysis.

DR. ISAAC H. RICHTER (Brooklyn, New York): The presence of Q waves in the electrocardiogram means

necrosis of the myocardium; however, it is possible that the Q waves appear in the early stages when the changes are still reversible. One of our patients, who died four hours after admission, came to autopsy. He was admitted to the hospital in shock and congestive heart failure, and there was not enough time to administer a sufficient amount of thrombolysin for evaluation. The autopsy findings revealed the presence of a recent myocardial infarction and a fresh thrombus in the right coronary artery. These findings in man cannot be compared to the experimental animal. In the latter the exact time of thrombosis is known, adequate amount of thrombolysin is given and the animal does not die of its coronary occlusion. A real test of comparison and evaluation would be the autopsy findings of those patients who received adequate amounts of thrombolysin, survived the acute attack of myocardial infarction and died at a later

At the present time we cannot come to any definite conclusions from our results, as this is only a preliminary report of a small number of cases. Our plan is to continue using thrombolysin in a large number of patients and compare the results to a similar number of patients receiving only anticoagulant therapy. This study must be pursued on a long term basis and evaluated as to the immediate mortality rate, symptoms of coronary insufficiency and survival rate in the two groups.

Treatment of Cerebrovascular Thrombosis with Fibrinolysin

Preliminary Report*

ROBERT M. HERNDON, M.D., JOHN S. MEYER, M.D., J. FREDERICK JOHNSON, M.D. and JAMES LANDERS, M.D.

Detroit Michigan

TREATMENT of patients with various types of L cerebrovascular disease has attained some measure of success in recent years. Antihypertensive drugs have been used successfully for hypertensive encephalopathy, anticoagulant drugs have been advocated for the prevention of cerebral thrombosis and embolism, 1,2 and the surgical removal of plaques in the cerebral vessels of the neck has been successfully performed for the treatment of patients with cerebro-vascular insufficiency.8-5 There is as yet, however, no effective treatment for patients with thrombosis of cerebral vessels which are inaccessible to the surgeon, or for patients whose general condition is unsuitable for surgical intervention. The latter patients comprise the great majority of patients routinely admitted to neurologic services.

The purpose of the present study has been to investigate the feasibility of the use of bovine fibrinolysin in proved cases of recent cerebrovascular occlusion. This study has been concerned with the evaluation of any dangers or potential dangers that might result from fibrinolysin therapy. Conclusions concerning its therapeutic effectiveness based on the present limited series would be premature.

MATERIALS AND METHODS

Chloroform-activated bovine fibrinolysin (Parke, Davis) was supplied to us in lyophilized form. This material was dissolved in 1,000 ml. of 5 per cent glucose solution in distilled water cooled to approximately 4°c. The solution was kept cold (as close to 4°c. as possible) by a refrigerant jacket during administration and was given intravenously over a three- to six-hour period. Initially, the solution was administered through a standard No. 20 gauge hypodermic needle; however, due to the local inflammatory reaction when the solution went perivenously, we now use a polyethylene catheter routinely for the purpose of intravenous infusion of the solution which has a cloudy grey appearance possibly due to some of the material remaining as a colloidal suspension.

Thirteen patients were selected who fulfilled the

following criteria:

1. All patients were acutely ill and showed some objective neurologic deficit clearly attributable to severe focal ischemia of less than seventy-two hours'

2. Lumbar puncture was performed on all patients and evidence of intracerebral bleeding (xanthochromia, erythrocytes exceeding 3,000 except when clearly attributable to a traumatic tap) was considered a contraindication.

3. A past medical history of allergic diseases was considered a contraindication.

4. Whenever possible patients with transient neurologic deficits (cerebrovascular insufficiency) were excluded.

5. Diagnosis was confirmed by autopsy in three cases and by arteriography in seven. The remaining three patients were considered to be too ill for arteriography and the diagnosis was made on clinical

All patients admitted to the neurology service with the diagnosis of cerebrovascular thrombosis or embolism who met the aforementioned criteria were given anticoagulants (heparin and Dicumarol®) and started on fibrinolysin therapy within a few hours of admission. Anticoagulant drugs were given with the fibrinolysin to prevent recurrent thrombosis immediately after the completion of fibrinolysin therapy. The fibrinolysin was given in three separate doses of 20 to 35 Loomis units/kg. of body weight over a threeto six-hour period on three successive days. During the period of administration the patients were ob-

* From the Departments of Neurology, Physiology and Pharmacology, and Pathology, Wayne State University College of Medicine, and the Neurology Department, Receiving Hospital, Detroit, Michigan.

TABLE 1
Summary of Data of Patients Treated with Fibrinolysin

Case No.	Diagnosis	Reaction	Days of Therapy	Clinical Result	Arteriographic Findings
1	Occlusion of branch of left middle cere- bral artery	Ecchymoses	2	Improved	Large plaque in left carotid artery at bifurcation
2	Occlusion of branch of left middle cere- bral artery	None	3	Improved	Moderate arterio- sclerotic changes
3	Occlusion of left mid- dle cerebral artery	None	3	Died from conges- tive heart failure	Not performed, post- mortem examina- tion was refused
4	Occlusion of branch of basilar artery, upper pontine area	Local venous tender- ness	1	Improved, died 3 wk. later from a gastrointestinal hemorrhage	Severe arteriosclero- tic changes
5	Occlusion of left mid- dle cerebral artery	None	1	Died, postmortem examination re- fused	Occlusion of left middle cerebral artery distal to as- cending fronto- parietal branch
6	Occlusion of branch of left middle cere- bral artery	None	3	Improved	Severe arteriosclero- tic changes with plaques in both carotid arteries and a narrow tor- tuous right verte- bral artery
7	Occlusion of basilar artery	Ecchymoses, hema- turia, rectal bleed- ing	2	Died on third day	Not done, postmor- tem examination was refused
8	Occlusion (probably embolic) of branch of left middle cere- bral artery	Local inflammation where intravenous infiltrated	1 +1	Improved	Atheroscerotic nar- rowing of intrace- rebral vessels (dif- fuse and severe)
9	Thrombosis of right posterior cerebral artery	None	3	Marked improve- ment in first 6 hr.	Narrow left verte- bral artery; ex- cellent filling in carotid system
10	Thrombosis of basilar artery	Questionable febrile reaction, tempera- ture 101°F.	Less than 1	Died 31 hr. after therapy	Postmortem exami- nation con- firmed thrombo- sis of the basilar artery
11	Thrombosis of left in- ternal carotid artery	None	3	Died	Postmortem exami- nation confirmed thrombosis of the left internal carotid artery
12	Occlusion of branch of right middle cerebral artery	Generalized urticaria	Less than 1	Improved	Not performed
13	Occlusion of branch of right middle cerebral artery	None	3	Improved	Marked stenosis of proximal portion of right middle cerebral artery



Fig. 1. Case 10. Fresh thrombus in basilar artery.

served at frequent intervals for signs of any untoward reaction and blood pressure, pulse, respiration and temperature were measured hourly.

Routine laboratory studies included the following:



Fig. 2. Case 11. Pons; perivenous pressure hemorrhages due to severe brain swelling following thrombosis of the left internal carotid artery.

hemoglobin, white blood count, differential leukocyte count, serum glutamic oxaloacetic transaminase, fasting blood sugar, plasma urea nitrogen, serum lipids and cholesterol levels and daily prothrombin times. Frequent Lee-White clotting time determinations were also performed.

Plasma fibrinogen determinations were performed before, during and after therapy on three patients for a total of five days of therapy. The procedure used was a modification of the Whipple, Hurwitz procedure as described by Ware et al.⁶

RESULTS

General Observations: The total series, to date, comprises thirteen patients (Table 1). Of these, five died and the remaining eight showed clinical improvement of some degree. It should be emphasized that only critically ill patients and those considered to be suffering from severe cerebral thrombosis or embolism were included in this series. Thus, the high mortality rate was to be expected, and should not be attributed to fibrinolysin therapy.

Necropsy Findings: Postmortem examination was performed on three patients; two of these patients died during the three days of therapy (Cases 10 and 11) and one died three weeks later from gastrointestinal hemorrhage and shock (Case 4). In all of these cases the patho-



Fig. 3. Case 10. Edge of infarct, parieto-occipital area in patient with thrombosis of the basilar artery, showing normal vascular congestion at periphery of infarct.

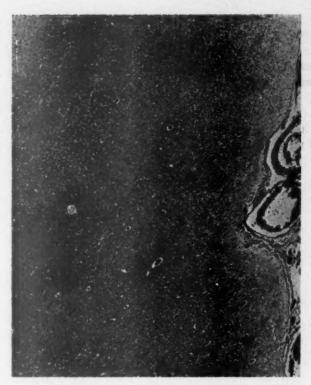


Fig. 4. Case 10. Occipital cortex, infarcted area showing ischemic infarction.



Fig. 5. Case 11. Temporoparietal cortex in patient with three day old occlusion of the left carotid artery. Ischemic infarction.

logic findings were of the type normally seen in cerebral ischemia due to thrombosis of cerebral vessels with ischemic infarction. In one patient (Case 4) who died three weeks after therapy with infarction in the vertebrobasilar system, no thrombus was found; however in one other patient (Case 10) who received only 250 units over a thirty-minute period (inadequate therapy) a fresh thrombus was found in the basilar artery (Fig. 1). In the third case in which postmortem examination was performed, a fresh thrombus was found in the carotid artery. This patient (Case 11) was also found to have perivenous hemorrhages in the pons (Fig. 2) which were not different from those frequently seen with acute brain swelling. The most important pathologic finding was that in none of the three patients treated with fibrinolysin was there any evidence of intracerebral hemorrhage or hemorrhagic infarction (Figs. 3 through 5).

Clinical Complications: Extracerebral hemorrhagic phenomena were noted in two cases. In one patient (Case 1) extensive ecchymoses of the skin and subcutaneous tissue developed in areas of recent trauma resulting from restlessness and confusion. This necessitated discontinuation of therapy after two days of treatment.



Fig. 6. Case 12. Patient with swelling about mouth and eyes following allergic reaction to fibrinolysin.

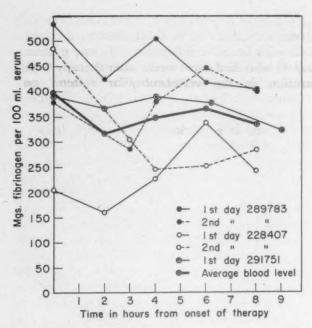


Fig. 7. Fibrinogen determinations during and after fibrinolysin therapy.

Subsequently the ecchymoses cleared rapidly. In the second patient (Case 7), who was believed to be suffering from thrombosis of the basilar artery with coma and quadriplegia, an area of ecchymosis developed over the left pectoral region on the second day of therapy and the patient was also noted to have microscopic hematuria. Therapy was discontinued shortly before completion of the third day, with no further enlargement of the ecchymotic area. The Lee-White clotting time when the ecchymosis occurred was twenty minutes and two hours later was forty minutes (resulting from depo-heparin therapy). The hematuria continued but did not increase in amount. minally the patient was noted to have rectal bleeding. Permission for necropsy was refused but on clinical grounds we concluded that the patient died from extensive infarction of the brain stem and that the bleeding did not contribute to her death.

In one patient (Case 12) generalized urticaria developed which necessitated discontinuation fifteen minutes after beginning the first injection (Fig. 6.). The patient responded rapidly to prednisolone therapy and no further complications occurred.

No severe febrile reactions clearly attributable to fibrinolysin were noted; however, in one patient with basilar arterial thrombosis an elevation in temperature of 101°F. developed which began thirty minutes after the start of

fibrinolysin therapy (Case 10). No other evidence of allergic reaction was noted. The patient died thirty-one hours later and postmortem examination confirmed the diagnosis of recent thrombosis of the basilar artery.

In one patient (Case 8) local erythema and tenderness developed, persisting for several days at the site where the solution was inadvertently injected perivenously. In another patient (Case 4) severe local tenderness developed along the vein of injection. For this reason, it was decided to discontinue treatment shortly before completion of the first day of therapy. No other evidence of inflammation was noted and the venous tenderness subsided during the succeeding twenty-four hours.

Delayed Sensitivity: Two patients, whose skin was tested for sensitivity to bovine fibrinolysin two to six months following therapy, showed sensitivity evidenced by an area of induration measuring 2 to 3 cm. at the site of intradermal injection of 1 unit dissolved in normal saline solution. In three of twelve untreated control patients areas of induration developed indicative of sensitivity. These results are in accord with guinea pig experiments which indicate that bovine fibrinolysin is roughly comparable to normal calf serum in its anaphylactogenicity.⁷

Arteriographic Studies: Arteriographic examination was performed prior to treatment in two patients and after treatment in five. Of the two patients in whom arteriograms were performed before treatment, one had an occlusion of the left middle cerebral artery and subsequently died of severe cerebral infarction. Occlusion was not demonstrated in the other patient although she had a right homonymous hemianopia, right hemiparesis and severe dysphasia. It was concluded that she had thrombosis of a small capsular branch of the left middle cerebral artery. Of the four patients who had arteriograms following treatment, all showed atherosclerotic or arteriosclerotic changes of various degrees but none showed complete occlusion of any vessel.

Laboratory Studies: Fibrinogen levels were determined in three patients before, during and after therapy and in two patients on two successive days. In each case an initial drop occurred followed by a rapid return toward initial levels after completion of that day's course (Fig. 7).

No significant changes were noted in hemoglobin, leukocyte count, fasting blood sugar, plasma urea nitrogen, or serum glutamic oxaloacetic transminase (except in one patient suffering from a recent myocardial infarction). Lee-White clotting time and prothrombin time were prolonged as a result of heparin and dicumarol therapy.

COMMENTS

The reactions we have observed with bovine fibrinolysin are avoidable in many cases. Skin testing prior to treatment (which we are now doing) should avoid allergic reactions. Occasional hemorrhagic phenomena (subcutaneous bleeding, hematuria and rectal bleeding) may not be avoidable always, but in general are not difficult to control and their danger has not seemed sufficient to prevent us from continuing investigation of this form of therapy.

The problem of sensitization is a more serious one. It is quite evident from our skin tests that sensitization to bovine fibrinolysin does occur and guinea pig experiments suggest that sensitization to normal bovine serum also occurs. This suggests that after a delay of three or more weeks a repeat course of therapy for subsequent vascular thrombosis may be potentially dangerous. However, before we were aware of this potential hazard we treated one patient with recurrent cerebral thrombosis with a second course of treatment three months after the first course without ill effect. In this patient long term anticoagulant therapy was not used. The effectiveness of fibrinolysin therapy remains to be determined. The drop in fibrinogen during the course of therapy indicates that there is a significant amount of activity present in the circulating blood during the course of the infusion. This, however, does not tell us whether or not the fibrinolysin is reaching the clot in quantities sufficient to cause thrombolysis and as yet we have insufficient data to state whether or not this treatment is beneficially modifying the natural course of cerebral thrombosis.

SUMMARY

- 1. Bovine fibrinolysin was used in the treatment of thirteen patients with cerebrovascular thrombosis.
- 2. Reactions were seen in six patients; none of the reactions were serious and many were avoidable.
- 3. Patients treated with bovine fibrinolysin may become sensitized to it and to bovine serum.
- 4. We conclude that intravenous bovine fibrinolysin is a feasible means of treating cerebrovascular thrombosis but that the present data are insufficient to permit conclusions concerning the clinical efficacy of this form of therapy.

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The Treatment of Cerebrovascular Thromboses and Embolism with Fibrinolytic Agents*

ROBERT L. CLARKE, M.D. and EUGENE E. CLIFFTON, M.D.

New York, New York

HUMAN fibrinolysin and streptokinase† have been shown to dissolve fresh intravascular thrombi in several pathologic situations.¹⁻³ These agents have been administered to extremely ill patients without serious side effects. Our results and those of others in treatment of patients with peripheral thrombophlebitis, peripheral arterial thrombosis and embolism, retinal artery and venous thromboses, coronary thrombosis, priapism and other conditions have already been discussed in this Symposium.

As indicated in a previous paper in this Symposium,⁴ our primary purpose to the present time has been to improve the materials available and to obtain satisfactory evidence that clinical improvement can be obtained in thromboembolic states. Our attention has now been directed to developing the most satisfactory methods of treatment and determining the situations in which a good response may be expected.

This paper presents the results of our experiences with patients with cerebrovascular thrombosis and embolism. It is obvious that cerebral thrombosis and embolism, and coronary thrombosis are among the most pressing clinical problems for investigation.

PROBLEMS OF FIBRINOLYSIN THERAPY

Treatment of patients with cerebrovascular thrombosis is difficult because other lesions may closely simulate it. The usual criteria for differentiating cerebrovascular thrombosis from cerebrovascular hemorrhage are far from infallible, although many clinicians now believe the differential diagnosis can be made satis-

† Merck Sharp & Dohme, West Point, Pennsylvania.

factorily. One of the dangers that must be faced is the possibility that the use of these agents in therapeutic amounts might aggravate a cerebrovascular hemorrhage.

In addition, the time element is also obviously important, if not critical, whether anticoagulants or fibrinolytic agents are used. The central nervous system tolerates poorly an acutely diminished circulation and a return of adequate circulation is imperative. Early accurate diagnosis is essential with the advent of clinically reliable fibrinolytic agents since they offer hope of return to normal. Perhaps, as has been suggested, the carotid angiogram will satisfy this need for early diagnosis.

CLINICAL RESULTS

We have treated only ten patients with cerebrovascular thrombosis or embolism. All were referred by their physicians after conservative treatment had failed. The clinical course of these ten patients is summarized in Table 1. Only one of the ten patients received treatment within six hours, which may be considered the upper limit of probable tissue survival, and unfortunately this patient was treated inadequately for an arterial occlusion according to our previous criteria. It was particularly rewarding to note that two patients showed rapid complete recovery and that three showed unexpected rapid improvement, one of whom (Case 1) had been observed for one month without improvement prior to fibrinolysin treat-

Only one patient had a severe reaction and this is difficult to evaluate properly (Case 2).

^{*} From the Research Laboratory of the Second Surgical (Cornell) Division, Bellevue Hospital, and the Clotting Mechanism Section, Sloan-Kettering Institute for Cancer Research, New York, New York. This work was supported in part by research Grants H4211 and 2867 from the National Institutes of Health, Bethesda, Maryland.

Table I

Clinical Data in Ten Cases of Cerebrovascular Thrombosis or Embolism

Case No.	Age (yr.), Sex	Associated Diseases	Type of Cerebrovascular Accident	Time Before Treatment	Duration of Treatment	Fibrinolytic Units	Results
			Systemic Instillati	on of Fibrinolyt	ic Agent		
1	74, M	Phlebitis; diabetes	Thrombosis	1 mo. (deep coma)	6 hr. 6 hr. 4 hr.	320,000 320,000 240,000	Improved; recovered from deep coma; able to communicate; no motor improvement
2	52, M	Rheumatic heart disease; aortic stenosis	Thrombosis	36 hr.	9 hr.	674,000	Improved; moderate motor improvement despite evidence after therapy of bloody spinal fluid
3	70, F	Previous stroke; thyrotoxicosis; kyphoscoliosis	Thrombosis	3 hr.	6 hr.	480,000	No change
4	57, M	Subacute bacterial endocarditis	Thrombosis	3 wk.	6 hr. a day for 4 days	450,000 for 4 days	Improved; aphasia cleared; hemiparesis lessened; death 48 hr. after treatment related to large meat bolus at carina and right main bronchus
5	78, M 81, F	Arteriosclerosis Auricular fibrilla-	Thrombosis Embolus	5 days 24 hr.	10 hr. 16 hr.	750,000 800,000	No improvement
7	54, M	tion Heart failure, au- ricular fibrillation	Embolus	12 hr.	131/2 br.	1,000,000	Complete recovery
			Local Instillation	n of Fibrinolytic	Agent		
8	45, F	Thyroidectomy	Postoperative throm- bosis of the common carotid artery	3 days	10 min.	200,000	Complete return of cir- culation; recovering but died 48 hr. later of hematoma com- pressing trachea; hep- arin and dicumarol given
9	45, F	Ruptured aneu- rysm of the in- ternal carotid arteries	Postoperative throm- bosis of common carotid artery	9 hr.	20 min.	275,000	Artery open; recovering reruptured aneurysm; died
10	61, M	Radical neck dis- section	Thrombosis of the com- mon carotid artery	12 hr.	1/2 hr. day 1-8 hr. day 2-8 hr. day 3-8 hr. day 4-4 hr.	275,000 400,000 400,000 400,000 200,000	Artery open; complete dissolution of throm- bus; circulation open

This patient was considered unsatisfactory for anticoagulant therapy because of severe hypertension and previous severe nosebleeds. He was accepted with reticence because of this contraindication. A course of twelve hours of therapy was planned, but after nine hours a nosebleed occurred. The infusion was stopped by the intern without notifying us or obtaining blood for our studies. We do not know if the bleeding was associated with excessive activity. The next day blood was obtained on spinal tap. Despite these episodes the patient later showed improvement which was better than expected.

Of the three patients treated by local instillation of fibrinolysin into the occluded vessels, all showed complete recanalization of the vessels with normal blood flow. One of these patients

(Case 8) showed good clinical response with relief of coma and motion of the paralyzed extremities but unfortunately was overtreated with heparin and dicumarol. Hemorrhage into the neck occurred which, combined with thick tracheobronchial mucus, resulted in tracheal obstruction, which was not treated in time by tracheostomy. At the time of tracheostomy the previously thrombosed artery was pulsating normally.

In one patient (Case 9) lysis of the carotid thrombus occurred with the administration of 200,000 units of fibrinolysin. The patient had return of function in the paralyzed extremity and definite clearing of coma. Shortly thereafter, however, the patient had a recurrent hemorrhage from the aneurysm for which the

original carotid ligation was performed. It seems only logical to expect lysis of the clot plugging the aneurysm as well as that in the carotid artery.

CASE REPORTS

Of the ten patients, four cases are presented in some detail.

CASE 4. A fifty-seven year old white man was admitted to the hospital with congestive failure. Two months previously he had been admitted elsewhere with congestive heart failure and subacute bacterial endocarditis. On physical examination he was found to have congestive failure. Findings were noted suggestive of rheumatic heart disease with aortic insufficiency, mitral stenosis and insufficiency. The spleen was not palpable and petechia were not present; however, because of the history, physical findings and his febrile course, the diagnosis of subacute bacterial endocarditis was made. At no time during his hospitalization was a positive blood culture obtained. Six days after admission he suffered a right cerebrovascular embolus or thrombosis with resultant aphasia and hemiplegia. The patient deteriorated rapidly. He was febrile (temperature, 103°F.) and comatose with Cheyne-Stokes respirations.

Three weeks following the cerebrovascular accident, because of the hopelessness of the situation as it then appeared and despite the lack of supportive evidence for subacute bacterial endocarditis, it was decided to treat this patient with fibrinolytic agents for unremitting subacute bacterial endocarditis. He was treated with 75,000 units per hour, six hours a day for four days with an adequate fibrinolytic response. His febrile course was not altered. The massive antibiotic therapy was continued. However, somewhat to our surprise the evidences of severe cerebral impairment lessened noticeably. The aphasia cleared and the hemiplegia lessened to a mild paresis. Thirty-six hours after the completion of the fibrinolytic therapy the patient was noted to have labored respirations and cyanosis and died

shortly thereafter.

Postmortem examination was limited to the thorax and abdomen. The heart and great vessels revealed only a small healing intimal tear in the ascending aorta. The right main stem bronchus was completely occluded by a bolus of meat and vegetable matter, measuring 5 by 3 cm. with distal atelectasis and early pneumonia. The patient's death after so remarkable an improvement was undoubtedly related to this bolus.

CASE 5. A seventy-eight year old white man was admitted to the hospital with an acute cerebral thrombosis of five days' duration as evidenced by right hemiparesis. On admission, blood pressure was 180/100 mm. Hg; respiration, 18 per minute; pulse, 80 per minute; and temperature, 37.8°c. Examination revealed grade II retinopathy and right hemiparesis.

The patient received 750,000 fibrinolytic units during the first two days of hospitalization and then anticoagulants (heparin). A physical medicine consultant two days after fibrinolysin therapy found slight difficulty with skilled movements of the hand as the only residual defect. The patient has continued to do exceptionally well.

Case 7. A fifty-four year old white obese man was admitted to the hospital with hypertension and congestive heart failure. His previous admissions to the hospital had been for congestive heart failure and pneumonia in 1957.

On February 17, 1959, he suffered a questionable pulmonary infarct as his rhythm changed from normal sinus rhythm to auricular fibrillation. On March 24, he was given quinidine and he reverted to normal sinus rhythm. Four hours later he was noted to have left hemiparesis. The diagnosis at that time was embolus to the right middle cerebral

At 8 P.M., twelve hours after the appearance of the hemiparesis which had remained unchanged, he was started on a course of intravenous fibrinolysin. He was given 1,000,000 fibrinolytic units in thirteen hours without ill effects. This therapy resulted in euglobulin fibrinolytic times of ten to twenty-four minutes. The whole clot lysis time went down to thirty-five minutes. The clotting factors stayed within normal limits except for a low prothrombin consumption of 66 per cent. The clotting factors the next day were relatively normal except for a slightly elevated Quick prothrombin time of 17.4 seconds. The patient made a most rapid recovery, losing all evidence of the hemiparesis within forty-eight hours.

Case 10. A sixty-one year old white man who had undergone left radical neck dissection suffered a severe hemorrhage from the left common carotid artery five days postoperatively. He was taken to the operating room where the common and internal carotid arteries were ligated. Five and a half hours postoperatively, he was noted to have hemiplegia and aphasia. He was given heparin nine hours after ligation and five hours after the onset of symptoms. Twelve hours after the ligatures had been placed, the patient was returned to the operating room where the ligatures were removed. The artery was closed by a firm thrombus which extended into the cranial portion of the artery. Over a halfhour period, 275,000 fibrinolytic units were instilled directly into the thrombosed artery. The vessels which had been pulseless without backflow demonstrated good pulsations and a brisk bright red backflow at the conclusion of the administration. An arteriogram was obtained which revealed a patent left cerebrovascular system (Fig. 1).



Fig. 1. Case 10. Arteriogram showing patent left cerebrovascular system following local instillation of fibrinolytic agent into occluded left carotid artery.

Following the return from the operating room with a patent arterial system, he was given 50,000 units per hour of a fibrinolytic agent eight hours a day for three days and for four hours on the fourth day. Fibrinolytic activity during both the local instillation and the subsequent four days reached adequate therapeutic levels. The patient showed only minimal functional improvement. The visual demonstration of what can be done with fibrinolytic agents in a freshly thrombosed artery was most gratifying. The next step will be to determine the circumstances necessary to insure functional recovery.

COMMENTS

We attempted in these first ten cases of cerebrovascular thrombosis to determine the best way to administer the clinically reliable fibrinolytic agents now available. We make no attempt to enunciate the future of fibrinolytic therapy in the treatment of these patients. Two factors appear to us to be particularly important. One factor is the place of the arteriogram in the early definite diagnosis of a cerebro-

vascular accident. If the physicians who are responsible for the care of these patients will agree that this procedure is the reliable method for definitive diagnosis, then we would suggest that an arteriogram be taken prior to fibrinolytic therapy. The other factor is how easily and quickly the occluded vessels are opened by direct instillation. In each of the three cases in which local instillation was attempted, the occluded vessel was opened and pulsating within a few minutes.

SUMMARY

 Ten patients with cerebrovascular thrombosis treated with fibrinolytic agents are presented, four in detail.

2. The arteriogram is essential for early diagnosis of a cerebrovascular thrombosis.

3. Local instillation of fibrinolytic agents into the occluded vessels is the most effective method of therapy.

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DISCUSSION OF PAPERS BY DRS. HERNDON, MEYER, JOHNSON, LANDERS; AND CLARKE AND CLIFFTON

DR. JACK P. WHISNANT (Rochester, Minnesota): I would like to present briefly some material obtained in collaboration with my colleague, Dr. Frank Howard, and others. We thought that our first step in approaching the problem of use of fibrinolytic agents in occlusive cerebrovascular disease was to determine whether they had any harmful effect on established cerebral infarcts in animals. We, therefore, produced cerebral infarction in animals by introducing small fragments of a clot into one internal carotid artery. This technic has previously been described by our group.

Twenty animals were given plasmin twenty-four hours after the infarction was induced. The preparation of plasmin contained 28,000 Christensen units per vial (prepared by Merck Sharp & Dohme).

Each animal was given approximately 60,000 units intravenously in one and a half to two hours. Five days after the infarct was produced, the animals were killed and each brain was sectioned coronally into seven slices. The area of infarction and the areas of hemorrhagic infarction were traced onto a mimeographed copy of drawings of the seven slices. The infarcts and the hemorrhagic areas were traced with a planimeter to obtain the surface areas in square millimeters. The percentage of the infarct which was hemorrhagic was thus calculated. In nineteen control animals cerebral infarcts were produced, but no plasmin was given.

By the end of the intravenous infusion of plasmin in an average animal the fibrinogen level in plasma was reduced from approximately 500 to 200 mg./100 ml., and the time required for lysis of the euglobulin clot was shortened from three and a half hours to approximately fifteen minutes. The time required for lysis of the blood clot was markedly reduced and the prothrombin time was significantly prolonged.

The extent of hemorrhagic infarction in the animals treated with plasmin was almost identical to that in the control animals. This study then indicates that with the amount of plasmin used no unusual or undesirable hemorrhagic changes occurred in the infarcts of the animals treated with plasmin.

Despite this rather optimistic note, as a clinical neurologist, I must introduce a note of pessimism. I am not entirely optimistic about the application of fibrinolytic agents in occlusive cerebrovascular disease. I cannot share the opinion that the patients with intermittent focal ischemic episodes should be treated with plasmin. We do have effective treatment for such patients, and long-term treatment with fibrinolytic agents does not seem feasible. Indeed, it probably would be harmful, as pointed out in previous papers in this Symposium.

Perhaps the most promising area of occlusive cerebrovascular disease that deserves careful consideration in regard to the effectiveness of fibrinolytic agents is progressing infarction or what has been called "slow stroke" or "stroke-in-evolution." This category of stroke is represented by the patient who is observed by his physician to have a gradually increasing neurologic deficit. If such a patient can be treated early and preferably by intra-arterial injection, one might well be able to show a favorable course compared with the natural history of the disease.

If a patient has had a recent cerebral infarct with only mild residual neurologic deficit and an appropriate arterial occlusion can be demonstrated, it is reasonable to expect a better long-term outlook for that patient if the occlusion can be eliminated by a fibrinolytic agent or by other means. However, I cannot believe that a significant change can be expected in the immediate course of a patient with a cerebral infarct simply by restoring the flow in the

occluded artery after infarction is established. I would emphasize then that in considering the treatment of patients with any aspect of occlusive cerebrovascular disease, it is exceedingly important to consider what one can expect from the natural history of the disease.

DR. MORTON LINDER (Valhalla, New York): Dr. Clarke, do you believe that direct instillation of the fibrinolysin material into the carotid artery is a preferred method in those patients with lenticulostriate arterial obstruction?

DR. JOHN S. MEYER (Detroit, Mich.): Dr. Clarke, what technic do you use for fibrinolysin irrigation in the carotid artery? Do you do it by hand injection or by a special pressure injection device? Also, what were the clinical results in the other cases after the carotid thrombus was lysed? We understand that the data indicated return of flow but what were the clinical results? I am a bit concerned about whether the dissolution of the clot might result in cerebral embolization. Also, was the opinion regarding restoration of flow based on angiograms after treatment or on palpation of the pulse? Finally, our clinical data are in agreement with Dr. Whisnant's statement regarding experimental studies. In our study which Dr. Herndon has described, pathologic examination of the brain in patients who died after fibrinolysin treatment clearly showed that hemorrhagic infarction does not appear to be a common complication of treatment. It was one of the things we feared might result from fibrinolysin therapy.

DR. JAMES A. EVANS (Boston, Massachusetts): I have been working in this field for a number of years in conjunction with our neurosurgeons; Dr. Poppen has had a large experience with angiography in cases of cerebral aneurysms. During those years the percentage of accidents was small; however, when we began to employ lysis therapy for some cerebrovascular lesions and wanted to classify them by means of angiography before and after treatment, we immediately were confronted with trouble which I think is to be expected. With this method, an irritating agent is injected into the carotid artery; such an agent does not carry oxygen and further dilutes the oxygen that is reaching the infarcted area and the region of collateral circulation about it. The first patient who was given lysis therapy for cerebral thrombosis died on the operating table. This program has been followed in three additional cases. One patient died following angiography, and fibrinolysin therapy in the two patients who did not die was of no benefit. We are discouraged and have lost the interest and cooperation of our neurosurgeons in this field.

DR. ROBERT M. HERNDON (Detroit, Michigan): In answer to Dr. Whisnant's questions as to the problem of hemorrhage into these cerebral infarctions we are concerned about this as Dr. Meyer has pointed out. Our three cases in which autopsy study was performed are obviously inadequate to determine

whether or not we will have hemorrhagic infarction following therapy; however, as was pointed out, we have not noted this complication as yet. I would agree that the cases of progressive infarction are probably ideal for the use of fibrinolysin. Another question, how often is a thrombus or embolus not the cause of a cerebral artery occlusion? I am not quite clear as to what is meant by how often is a thrombus or embolus not the cause of occlusion of the cerebral artery. In my experience, thrombosis and embolism are at least the most common causes of occlusion of the cerebral artery. However, arterial occlusion is not always the cause of infarction.

In many cases of cerebral infarction, no arterial occlusion can be demonstrated arteriographically, and the infarction appears to be due to arterial insufficiency resulting from stenosis of a vessel supplying the area involved in the infarct.

DR. ROBERT L. CLARKE (New York, New York): We administered the material directly into the carotid artery. This is performed by exposing the artery through a sternomastoid incision and placing

a polyethylene catheter directly into the artery and instilling the material. Incubation for fifteen to twenty minutes rather than irrigation is the underlying principle. We know that the vessel opened after treatment by relying principally on our clinical impression of a vessel which before treatment is pulseless and firm, from which we cannot withdraw blood through a large bore needle, and which after treatment becomes softer, bright red, with an expansile pulse from which we can easily draw arterial blood. In those cases in which it seemed appropriate in regard to the patient's over-all condition, we have also obtained an arteriogram. We want to make perfectly clear that we in no way are trying to evaluate our results as to eventual functional recovery or the advantage or disadvantage of using these agents in people with cerebrovascular thrombosis. Our only purpose was to advise those people who will set up the control series on strokes as to what we think is the best method of approach for this particular type of problem.

Panel Discussion: The Evaluation of Clot-Lysing Agents in the Treatment of Thrombotic Disease

Moderator: Joseph E. Sokal, M.D., Buffalo, New York Sidney P. Hecker, M.D., Palo Alto, California Alan J. Johnson, M.D., New York, New York Richard Warren, M.D., Boston, Massachusetts

DR. SOKAL: Our discussion will focus on the use of thrombolytic agents in the treatment of thrombotic disease in man. This panel is composed of four clinicians, all of whom have had experience in the treatment of thrombotic disease and all of whom have used thrombolytic preparations. Dr. Sidney P. Hecker, Dr. Alan J. Johnson and I are internists. Dr. Richard Warren is a surgeon.

We will not attempt to summarize the wealth of material that has been presented in this Symposium. Instead, we will discuss some of the problems that confront us as fibrinolytic therapy enters the phase between experimental development and acceptance as part of medical practice. Thromboembolic disorders are the principal causes of mortality and serious morbidity in this country. What promise does fibrinolytic therapy offer us that this toll can be reduced? In which conditions are fibrinolytic agents likely to be effective? Will we reverse the pathologic process in 20 per cent of treated patients or in 80 per cent? How do we select patients for treatment with fibrinolytic agents? How do we decide that thrombolytic therapy was really responsible for improvement when such has occurred? How do we determine dose requirements and how long do we treat? What about anticoagulants? Finally, are we ready to Pecommend fibrinolytic therapy for general use or is this still a subject for research in our major investigative centers?

First of all, let us consider some of the diseases in which fibrinolytic therapy might be used. I shall ask our panel members to list the conditions for which, in their opinion, there is good evidence of the effectiveness of fibrinolytic therapy.

DR. HECKER: There is much experimental and clinical evidence that we have effective agents for dissolving fresh fibrin clots in all parts of the vascular tree. Thrombophlebitis has been the best studied entity, in terms of number of patients treated and reported on. This condition has also had most of the clinical successes, although some of these are open to question because of the methods used in the evalua-

tion. Evidence is available that in vascular surgery, plasmin can reopen freshly thrombosed anastomoses or dissolve a secondary thrombus which has formed distal to an arterial embolus.

It is surprising that successful treatment of thrombosis of the retinal artery, which should be an ideal situation in which to evaluate the lysis of small, fresh clots, has not yet been reported. Howden described one patient with thrombosis of the central retinal vein who rapidly recovered excellent visual acuity following plasmin therapy. I would like to show one instance of partial central retinal artery occlusion in which we believe a dramatic result was obtained (Fig. 1).

The patient is a sixty-five year old man with diabetes which was well controlled without insulin who had momentary episodes of blindness for a month prior to his final retinal occlusion. When he suddenly became blind one day, he was admitted to the hospital and treated with anticoagulants, retrobulbar administration of Priscoline,® and orally administered nicotinic acid. Anterior chamber decompression was performed twice and stellate ganglion block once during the following two days. Despite these measures, visual acuity diminished from the ability to count fingers at 3 feet on admission to counting fingers at 1 foot three days later. At this time an infusion of plasmin (thrombolysin) was administered for six hours. Within ninety minutes after the onset of infusion the patient volunteered that he could see figures on a television screen 10 feet away and he was able to count fingers at 6 feet. The first fundus photograph (Fig. 1A) revealed the pale grayish, ischemic retina with early exudate and edema near the optic disc. The second picture (Fig. 1B) was made two days later. The retina had a normal pink color, and the vessels were better seen because the edema had greatly subsided. By this time his visual acuity was 20/100 and he could count fingers at 10 feet. The final picture (Fig. 1C) was made three weeks later. The exudates are almost resolved, pallor and edema are absent. The patient's visual

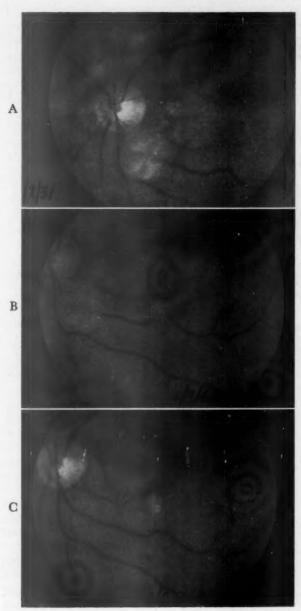


Fig. 1. Partial occlusion of the central retinal artery treated with plasmin infusion. A, fundus photograph before treatment showing ischemic retina with exudate and edema near optic disc. B, two days after treatment, showing more normal color and subsidence of edema. C, three weeks later; exudates almost completely resorbed, and pallor and edema have disappeared.

acuity when last examined was 20/40 and he can take care of himself very well. Anticoagulant therapy is still being carried on.

Dr. Sokal: How about such conditions as arterial thrombosis and pulmonary embolism?

DR. HECKER: The number of patients with pulmonary embolism reported to have benefited from plasmin therapy are really few. Sheffer and Israel reported that four of eight pulmonary infarcts underwent rapid resolution as visualized by serial films of the chest within a few days whereas usually, roentgenographic signs persist for over a month. However, these are few patients. There have been a few other reports that describe remarkable clinical improvement immediately after fibrinolysin administration. This may also be obtained with heparin. However, in a number of instances, despite "adequate" heparin therapy, patients with repeated pulmonary embolism cease throwing emboli after plasmin is used. I have seen one such case. The value of plasmin, however, is not yet adequately established to recommend clinical use in all patients with pulmonary embolism.

DR. WARREN: There are several clinical conditions in which clots have been dissolved. However, those I intend to list have no connection with whether or not I would use thrombolysin in their treatment. This is because evidence for clot lysis is difficult to obtain as shown in many of the papers presented in this Symposium. In our experience with seventy-five infusions in approximately fifty patients we have not been able to prove that we have entirely lysed a clot in any patient. The diminution of a clot has been obvious in some patients with superficial phlebitis, but the complete opening of a vessel has not been observed. But in these fifty patients there have been perhaps only six or seven in whom we have had tools with which to evaluate whether or not this has happened. However, the evidence of others shown in this Symposium indicates that clots have been dissolved in patients with superficial phlebitis, in some of the patients with acute thrombosis of the carotid artery reported on by Dr. Clarke (p. 546) and in some of Dr. Evan's patients with thrombosis of the axillary artery following radical mastectomy (p. 550).

Dr. Johnson: This is a difficult question because of the qualifying adjective, "good evidence for the effectiveness of fibrinolytic therapy." We have good evidence for a beneficial effect in the treatment of thrombophlebitis. I am not sure, however, that this effect is due solely to a specific proteolytic effect. Prior to using the defined system for induction of proteolysis, which we described previously, only a small proportion of the patients with thrombophlebitis treated by less well controlled methods showed unequivocal clot lysis. If the clots are not too old, and the defined system used later is also used in the treatment of these patients, consistent clot lysis could be produced. To return to Dr. Sokal's question, we have good evidence for a therapeutic effect in the case of thrombophlebitis. This effect may or may not be accompanied by a thrombolytic effect. The thrombolytic potential is certainly present, however.

DR. SOKAL: I would agree that we have good evidence of therapeutic effectiveness in acute thrombophlebitis and in certain types of arterial thrombosis. I was impressed by Dr. Boucek's presentation (p. 525) of his work in perfusing the coronary arteries with thrombolytic agents. The evidence for effectiveness in coronary thrombosis is more convincing to me now than I thought it was before this conference.

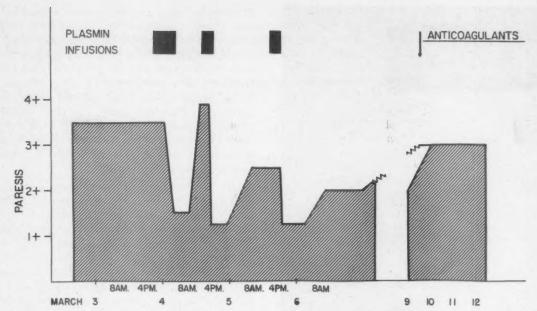


Fig. 2. Cerebral thrombosis treated with plasmin. Average motor and speech loss in involved areas is represented by the height of the shaded area. Note the improvement following each infusion of plasmin and relapse some hours after completion of treatments.

On the other hand, I would be loath to commit myself on the subject of pulmonary embolism; most patients either die before thrombolytic therapy could be effective or recover without it.

Some of the effects observed after administration of thrombolytic agents do not appear clearly related to the dissolution of major clots. They might be categorized rather as "anti-inflammatory" effects. Dr. Johnson, would you comment on the so-called "anti-inflammatory" effect of fibrinolytic agents?

DR. JOHNSON: The term "anti-inflammatory" is not a good one. The possibility that these enzymes might exert an anti-inflammatory effect has intrigued us for many years. Although previous publications have described such an effect, the evidence has been largely clinical. Experimental evidence for this has been primarily limited to the work of Gordon and Ablondi in rabbits. Our unpublished experimental evidence in man seems to indicate that there is an anti-inflammatory effect. The clinical features of this effect were evident some years ago, when patients with large amounts of streptokinase antibody and long standing, unresponsive thrombophlebitis were given small amounts of streptokinase (15,000 to 20,000 units). The redness, induration and edema seemed to subside markedly in these patients although there was no measurable proteolysis in the circulating blood, and no clinical or radiologic evidence for thrombolysis in the major vessels. Thus, we thought the clinical effect in these patients must be due to a mechanism which was separate and distinct from fibrinolysis or thrombolysis.

DR. SOKAL: Are there any further comments on the anti-inflammatory effects of plasmin?

DR. HECKER: We have been impressed with the

observation that evidence of inflammation often subsides promptly, even though we cannot subsequently demonstrate that clot lysis has occurred. Symptoms and signs of inflammation may disappear within a few hours after the infusion begins. One of our patients was in the hospital with severe active rheumatoid arthritis when a deep thrombophlebitis developed. She was treated with plasmin. During the infusion, all symptoms and signs of involvement of the joints subsided completely. For the first time in weeks she was able to move all her joints comfortably. Within a few hours after the infusion was stopped, they gradually stiffened again and pain returned. During the second infusion, a lesser but similar effect was noted. Perhaps a fibrin-rich exudate in the joints was being dissolved. but the anti-inflammatory effect was most dramatic.

DR. WARREN: In trying to evaluate thrombolytic effects, we must be aware of these non-specific effects which seem to occur in some patients. We should not interpret everything we see as evidence of clot dissolution.

DR. SOKAL: This is a good point and we will have some evidence bearing on it later.

I doubt that one case constitutes good evidence, but it might be of interest to discuss the results in one patient with a cerebral vascular accident. This fifty-two year old man suffered a stroke twelve to twenty-four hours before treatment was started. His course is shown in Figure 2. When he was admitted to the hospital, he was aphasic and had right hemiplegia. About five hours after the start of the first plasmin infusion, he began to speak and motion in his arm and leg returned. His average motor loss decreased from almost complete paralysis to mild

paresis. For reasons that are not germane to this discussion, anticoagulants were not administered. The next morning his motor loss was somewhat greater than it had been on admission. A second plasmin infusion was given and again he had a dramatic return of speech and motion. During the sixteen hours after the termination of the second plasmin infusion, his condition again became worse, although not to the initial degree of motor loss. After the third plasmin infusion, there was again a rapid improvement in function, followed once more by deterioration soon after cessation of therapy. Anticoagulant administration was finally started six days after admission, but no improvement was seen at this time.

I would not offer this case as evidence for the usefulness of fibrinolytic therapy were it not for the striking coincidence between the three periods of treatment and the three periods of improvement, followed by relapse after cessation of each treatment. We believe that these events are consistent with the hypothesis that thrombi were lysed by the plasmin infusions and that thrombosis recurred in damaged vessels some hours after the end of each treatment. With two or three more cases similar to this, I would be willing to claim that we have good evidence of the effectiveness of thrombolytic therapy in strokes.

In what situations would you use fibrinolytic therapy? This differs from the topic we have just discussed. There might be situations in which you are sure of the effectiveness of fibrinolytic therapy, but might elect not to use it. We do not give insulin to every diabetic patient, for example. On the other hand, there are situations in which you are not convinced that fibrinolytic therapy is effective, but where you might want to try it because of the seriousness of the case.

Dr. Warren: Although I am sometimes considered a pessimist, in this respect I am definitely an optimist. Certainly the experimental evidence presented in this Symposium shows that there are many experimental situations in which these fibrinolytic materials dissolve clots. In clinical situations it is difficult to appraise the various factors and determine the proper dosage. These factors are the access of the material to the clot or clots and their size and age. Since the use of the materials is still experimental and probably will continue to be so for some years, such considerations must determine which patients to treat. We are interested in treating patients with acute phlebitis, those with thrombosis of the retinal artery, and those with massive pulmonary embolism. I refer to a small group of patients with massive pulmonary embolism who have survived, but in whom the embolism is only a few hours old and is still causing embarrassment of the circulation and right heart failure. These patients have a fresh clot which is not in a part of the vessel in which there is disease. It is logical to assume that we will be able to deliver the material to the clot and procure enough clot lysis to

make the difference between survival and death. At this time I am not interested in using this therapy in patients with severe arteriosclerosis obliterans whose thrombus has been the final episode in the total closure of the artery. I have seen many arteries at surgery which have been narrowed to 2 or 3 mm, in diameter and in which the clot is the agonal episode of the total closure. In such vessels it seems almost impossible to deliver the fibrinolytic material to the clot or to keep the clotted area open after it has been lysed. In these situations I include coronary occlusion and arterial thrombosis in other arteries superimposed on arteriosclerosis. In most clinical situations the patient is not seen until at least three hours after the thrombosis. Arterial embolism also would not be amenable to therapy because the embolic material is almost always of some age before it breaks off and lodges in its embolic site. I did omit one other indication: arterial thrombosis which occurs on the operating table during arterial surgery. When operating on arteries we are interested in using thrombolytic materials locally at that time.

DR. JOHNSON: I would like to emphasize strongly that this is a period of experimental and clinical investigation. Thus, when it can be clearly established that a fresh thrombus has caused disease, and a well defined thrombolytic agent is available, utilization of this agent will benefit both the patient and the investigator.

Dr. Sokal: I, too, would use thrombolytic agents in serious acute thrombotic episodes even if I were not convinced that we had good evidence of therapeutic effectiveness in such situations. On the other hand, I would not use thrombolytic agents in all cases of acute thrombophlebitis, even though this is the area where we have the best evidence of effectiveness. I would reserve thrombolytic therapy for elderly, debilitated and postoperative patients in whom the hazard of pulmonary embolism is high. I would also use it in patients who already have a compromised circulation in an extremity. However, I have not recommended fibrinolytic therapy for young vigorous persons with relatively mild thrombophlebitis because I believe that these patients handle their disease very well even with minimal treat-

DR. HECKER: I agree with you entirely but would add one additional comment with regard to selection of patients with thrombophlebitis for thrombolytic treatment at the present time. The majority of young, otherwise healthy patients with deep thrombophlebitis of the calf do well with standard anticoagulant therapy. Iliofemoral thrombosis, on the other hand, frequently results in a postphlebitic syndrome even though anticoagulants are given. I would therefore suggest that iliofemoral thrombosis should be treated with plasmin, although there is little evidence at this time that this will successfully prevent the complication. In addition, even though plasmin

should still be used primarily as an experimental drug, the gravity of an illness such as hyaline membrane disease of the newborn would prompt me to consider its use in this condition. The best therapy for hyaline membrane disease, prior to the availability of fibrinolytic agents, has been prayer. There is some rationale and some early experimental evidence to suggest that these agents may offer the first positive approach to treating this condition. They warrant a trial either by aerosol or parenteral administration.

DR. JOHNSON: Dr. Hecker, I have been told by pediatricians that it is difficult to tell whether an infant has hyaline membrane disease until the patient has had progressive, grave respiratory distress for over twenty-four hours, and that the final diagnosis is a pathologic diagnosis.

DR. HECKER: This is a difficult diagnosis to make early. Usually the signs appear within a few hours after birth. Many pediatricians would essay that diagnosis within six or eight hours, if it was a premature baby or the result of a cesarean delivery or if there had been bleeding antepartum during the delivery. In all of these conditions hyaline membrane disease is more common. I admit that the syndrome of acute respiratory distress can be caused by other conditions in the premature newborn and often the diagnosis is not certain. If one waits for two or three days to make a definite diagnosis, the child is moribund. The treatment, even if started within twelve to twenty-four hours after birth, might still save some children.

DR. SOKAL: How do we decide in an individual patient whether or not fibrinolytic therapy has been effective? This is another difficult problem. Figure 3 shows the venograms of a patient with a deep venous thrombosis in the leg. The first film (A) was taken before treatment. There is a little dye in the collaterals but no dye in the central vein. This patient had a good clinical response to therapy. In the second film (B) you see a good demonstration of the opening up of the thrombosed vein. Figure 4 shows the venograms in a patient who had an even more dramatic clinical response, with all signs of thrombophlebitis disappearing within forty-eight hours after the start of therapy. However, the venograms show no lysis of the clot at all; in fact there is some retrograde extension of the original clot (Fig. 4B). However, flow through the collateral vessels has appeared. Therefore, this patient, who had a dramatic clinical response, actually had propagation of the clot during therapy. Her improvement is undoubtedly related to opening of collateral channels and perhaps also to the "anti-inflammatory" effect we have discussed.

DR. HECKER: Since the advent of anticoagulants, their effectiveness in the treatment of thrombophlebitis has been well demonstrated. In recent years, a number of additional agents have also been employed. These are either non-specific proteolytic enzymes such as trypsin, or anti-inflammatory mate-



Fig. 3. Acute thrombophlebitis treated with plasmin. Excellent clinical and philebographic response. A, phlebogram immediately before therapy. B, phlebogram on the fifth day. Main venous channel is open and flow through collaterals has decreased greatly.

rials such as Butazolidin. Both of these are reported to improve the clinical course of thrombophlebitis. Innerfield believes the clinical response is better with trypsin than with anticoagulants. All these agents differ in their mode of action yet all produce clinical improvement. What does this mean in evaluating fibrinolytic substances in the treatment of thrombophlebitis? It means that it would be dangerous to assume that a good clinical result is actually due to thrombolysis. Sokal, Ambrus and Ambrus showed two years ago that there could be discrepancies between external objective evidence of improvement and the venographic evidence of the presence of a clot.

We have recorded serial venograms in a number of patients in an effort to correlate the external appearance with what is inside the vein and to see how often clot lysis actually occurs. We have found that even the diagnosis of the presence of a clot is often equivocal, and that even when good external evidence is present, there may be no clot in the vein. This certainly will confuse the evaluation of a thrombolytic drug if venographic evidence is not available. One

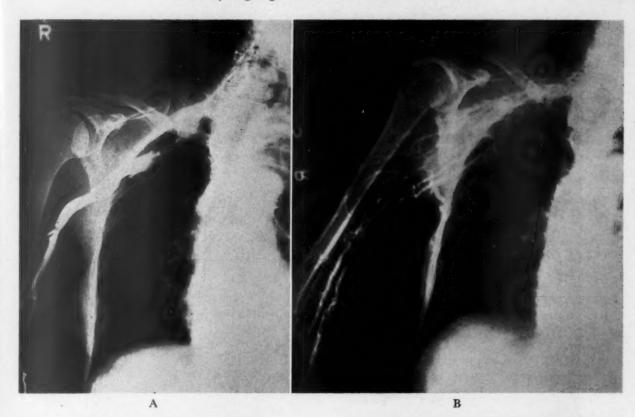


Fig. 4. Acute thrombophlebitis treated with plasmin. Excellent clinical response; propagation of thrombus by phlebography. A, phlebogram immediately before therapy shows complete obstruction of axillary vein and no filling of collaterals. B, phlebogram four days later, after complete clinical recovery, shows retrograde propagation of axillary vein thrombus; blood flow through collaterals.

patient (Fig. 5) had acute onset of calf pain in the right calf while playing basketball. He had all the physical findings that are classic for deep thrombophlebitis. Yet his venogram is normal (Fig. 5A). Because of this, he was not given treatment. Two days later, the diagnosis became apparent (Fig. 5B). Ecchymosis appeared in the calf and ankle. He had apparently ruptured a muscular vein while playing. There were no complications. Other patients have been seen with typical physical findings of thrombophlebitis but normal venograms. They have not been treated and have had no sequelae.

Confusion may also develop because of an antiinflammatory property of plasmin (Fig. 6). This patient had signs of phlebitis developing three days prior to treatment. The first picture (Fig. 6A) shows the block in the superficial femoral vein. Within several hours after the onset of plasmin therapy pain and tenderness disappeared completely. The post-treatment venogram, recorded forty-eight hours later (Fig. 6B), reveals that the block is essentially unchanged.

In Figure 7 are demonstrated the venograms of a patient with rheumatoid arthritis and deep thrombophlebitis. In the initial film (Fig. 7A), notice the paucity of veins in the calf. In the post-treatment venogram (Fig. 7B), notice the increased number of venous channels in the calf. How is this interpreted?

Were all these veins thrombosed or were these veins in spasm? Are they open now because of lessened spasm? If so, has administration of heparin or plasmin lessened the spasm? Notice the tubular narrowing in the post-treatment film of the popliteal vein, which is evidence of some persisting spasm. We know this is spasm because the position of the narrowed area changed in serial films.

The rapid development of collateral circulation and the use of tight bandages and elevation may also bring about rapid clinical improvement even in the presence of persistently blocked veins. This is well demonstrated by the case of a patient who had a thymic cancer removed and grafts inserted to replace his innominate veins and superior vena cava (Fig. 8). The grafts thrombosed, producing the superior vena cava syndrome seen in Figure 8A. Figure 8B, was made two days after plasmin treatment, and shows that the head has decreased in size but the arms are larger. Figure 8C was made three weeks later after ace bandages and elevation had been used on the upper extremities. The edema is gone. The important observations here were in the serial venograms (Fig. 9). The first one (A) was made at the start of treatment. The right axillary vein is occluded and there are some collaterals across the wall of the chest. The second film (B) was made at the time the patient



Fig. 5A. Traumatic rupture of muscular vein of leg simulating deep thrombophlebitis. Normal venogram on admission.

appeared entirely normal. It shows no reopening of the blocked channel.

These observations illustrate the extreme difficulty of drawing conclusions without angiographic evidence about what is happening inside an affected vein. The anti-inflammatory and antispastic effects of plasmin may confuse the observer who relies entirely on external clinical criteria. Clinical studies which have not employed serial angiography in the evaluation of thrombolytic agents in phlebitis leave the basic question of how many clots dissolve unanswered.

DR. SOKAL: This is a rather frightening demonstration of the pitfalls in the evaluation of therapeutic results in an individual patient. Dr. Hecker and I have selected these cases to point up the problem. These inconsistencies are not found all the time.

DR. JOHNSON: I would like to add just another word of caution: Use rigid criteria in evaluating fibrinolytic therapy. In addition to the venograms, therefore, I would like to include (1) long periods of observation prior to enzyme treatment (when possible) as an intrinsic control, and (2) double-blind studies on large numbers of patients. I am sure that these measures are being taken at the present time.



Fig. 5B. Same patient as in Figure 5A. Ecchymosis in calf and ankle two days after admission.

DR. WARREN: I would like to draw attention to the capriciousness of phlebographic evidence. There is a difference in this respect between phlebography and arteriography. If arteriography is proper in dosage and timing, a representative picture of the arterial tree is obtained, because all the arteries that are open in a given area will fill; whereas in the venous system the alternate pathways are so common that often the dye may take an alternate course. One must also keep in mind the possibility of spontaneous lysis which has been referred to in this Symposium. Dr. Robertson of Montreal has shown the spontaneous disappearance of clots placed in portal veins of dogs in an attempt to cause portal hypertension. Recently, I had an example of how arteriography might be misinterpreted. Our cardiologist, Dr. Littmann, procured an arteriogram on a young patient with angina pectoris which showed complete blockage of the left anterior descending coronary artery. I tried to persuade Dr. Littmann to allow surgery but he did not and six months later the repeat arteriogram showed that the artery had recanalized. This film could have been shown as evidence of surgical success had the patient been operated on.

DR. SOKAL: Regarding technic of therapy, what are your opinions about the use of anticoagulants in association with fibrinolytic therapy?

DR. Johnson: Heparin is valuable if used with sufficient caution. The value of heparin, as demonstrated by the earlier experiments of Wessler, obtain in this situation, as well as elsewhere. I would also like to emphasize the need for caution since the anti-

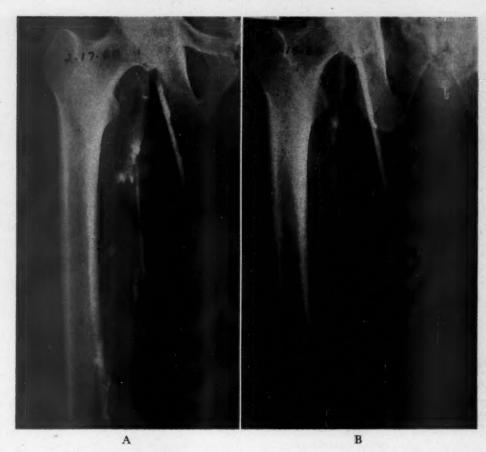


Fig. 6. Thrombophlebitis of superficial femoral vein. A, venogram prior to treatment, showing block in superficial femoral vein. B, venogram forty-eight hours after plasmin therapy shows block to be unchanged despite disappearance of pain and tenderness.

thrombin action of heparin may be potentiated by the antithrombin effect of the products of fibrinogen degradation, as previously shown by Sherry and Fletcher. In addition, there is often a decrease in the inhibitors to fibrinolysis as a result of the heparin injection, which increases the effect of the infused enzymes. Finally, heparin is probably better than the bishydroxycoumarin compounds because it probably acts at the place we want it to act and may be controlled from minute to minute, if necessary.

Dr. Sokal: Do you recommend concurrent anticoagulant therapy?

DR. WARREN: Yes.

DR. SOKAL: Dr. Ambrus and I saw recurrence of thrombosis in many patients in our original group who did not receive anticoagulants. We believe that fibrinolytic therapy should be followed immediately by administration of heparin.

DR. HECKER: We treat all patients who receive fibrinolytic agents with heparin between infusions and with prothrombinopenic agents thereafter. Although there is some evidence to suggest that heparin may be inhibitory to fibrinolytic agents in high concentration, I doubt that we ever reach this level in clinical treatment.

Dr. Sokal: How long should we administer

fibrinolytic agents? In our initial studies, Dr. Ambrus and I treated patients for three days. This was carried out because we were not using anticoagulants and because we wanted to be sure that we obtained a maximum effect. However, now that we all use heparin in association with fibrinolytic therapy, do we have to treat a patient for prolonged periods? This is difficult for the patient, the physician, and everyone else concerned. It seems to me that either we lyse the clot or we do not. If we administer a therapeutic dose of fibrinolysin within a few hours and then follow with administration of heparin, why must we continue intravenous infusions for twenty-four-hour periods or treat for many days?

DR. WARREN: We have done little in attempting to determine whether or not the thrombolytic activity persists in the clot following cessation of therapy and disappearance of evidence of activity in the peripheral blood. The work of Ouchi during the past few months has indicated that large doses of about 4,000 units of thrombolysin per kg. per hour are necessary in experimental clots. This, of course, is a tremendous dose, being about 250,000 units per hour in an adul. We have hesitated to use such large doses clinically. Dr. Ouchi has used about 100,000 units per hour in the last twelve patients, with continuous infusion of



Fig. 7. Rheumatoid arthritis and deep thrombophlebitis of veins of calf. A, pretreatment venogram. B, post-treatment venogram showing opening of venous channels.

from eight hours to thirty-nine hours. This has produced a four plus whole blood lysis test in the peripheral circulation in about a quarter of the patients, although in half the patients there has been some evidence of whole blood peripheral activity. In the patients in whom four plus peripheral activity developed in the whole blood lysis test, all had a marked drop in fibrinogen and in prothrombin. We have noted no hemorrhagic phenomena associated with these high dosages and high levels but we know that the patients will bleed if they have an open wound. Having now established this as a major dose, we are going to decrease it to a point where we can see if we can procure clinical effect without an effect on the other proteins.

DR. JOHNSON: We have to consider the origin of the clot as has been mentioned earlier in this Symposium. When a clot has been formed in an inflamed vessel, it is generally resistant to lysis; therefore, we have to spend more time lysing it. As demonstrated, nearly forty-eight hours of a high activator system were required to lyse a forty-eight-hour clot in one patient, although the whole blood clot lysis occurred in two to four hours and the euglobulin clot lysis time varied between ten and twenty minutes. Furthermore, this patient had his own constant infusion going on at an ideal level.

When we attempted to prevent reformation of clots by prolonging the SK infusion, after observing clot lysis in the superficial veins, we found only four to



Fig. 8. Superior vena caval obstruction secondary to thrombosed grafts of superior vena cava and innominate veins. A, before plasmin therapy. B, two days after treatment showing decrease of facial edema but persistence of edema of the arm. C, three weeks after treatment with ace bandages and elevation of arms, showing disappearance of edema of the arm.



Fig. 9. Vendgrams of same patient as in Figure 8. A, at onset of treatment showing right axillary vein occlusion. B, three weeks later when patient looked normal. Axillary vein is still blocked.

six hours of continued infusion were required to prevent reformation of the clots which had lysed. It took then, in these twenty-four- to thirty-six-hour infusions, somewhere between twenty and thirty hours to lyse these "resistant" clots.

I would like also to emphasize once again the relative resistance of older clots to lysis, although this has been stated many times previously. Unfortunately, in clinical situations, we cannot date the age of the clot; therefore, we must treat for prolonged periods as a matter of policy.

periods as a matter of policy.

DR. Sokal: The use of heparin will solve part of this problem.

DR. Johnson: The concomitant use of heparin might shorten the period of infusion in our own situation by only four to six hours of the thirty-six-hour infusion. In spite of this definitive evidence for a prolonged infusion, we cannot overlook the evidence presented here regarding local instillation, and the rapid clot lysis which seems to have been achieved by this method.

DR. SOKAL: I was impressed by the demonstration in this Symposium that a thrombolytic agent could be administered to an infarcted brain and not cause hemorrhage. This is reassuring. What do you consider to be the major potential dangers of fibrinolytic therapy?

DR. WARREN: With the new thrombolytic preparations we are not concerned with pyrogenic reactions. Fatients who receive large doses have had no more frequent or severe pyrogenic reactions than those who have received smaller doses. The important consideration when we find the exact dosage is the highest effective dose that can be administered without interfering with the fibrinogen.

DR. SOKAL: What do you watch for?

DR. HECKER: Reactions are relatively few in thrombophlebitis. A good clinical effect can be

achieved most of the time without much hazard. I have seen significant hemorrhage in only one patient who was inadvertently given heparin just at the end of her plasmin infusion while still oozing from the cutdown. There was a large hemorrhage in the subdeltoid region. I have no experience in the treatment of cerebral thrombosis, but I am worried about the use of thrombolytic agents in this condition. I am not convinced that these agents will not convert anemic infarcts into hemorrhagic ones.

DR. SOKAL: What precautions do you take during therapy?

Dr. Johnson: Whenever trauma occurs, bleeding can be expected to occur with these agents. This after all, is what we are trying to use them for, to lyse clots. With this in mind as a starting point, the best safeguard appears to lie in making those measurements which help in the regulation of dosage, therefore obviating those dangers which may arise from too high or too low a dosage. Thus, the determination of plasma fibrinogen may indicate excessive proteolysis of other plasma constituents. The determination of one-stage prothrombin time is very useful also, since it gives us a great deal of clinical information about the antithrombin effect of fibrinogen degradation products, the amount of factor v and possibly factor vII. If the fibrinogen and prothrombin time are carefully controlled, the infusions may be given with relative safety and a maximum thrombolytic effect obtained.

DR. SOKAL: What is the present status of thrombolytic therapy and what lies ahead? In a preliminary discussion, we agreed in our answer to this question and I have been nominated to speak for the entire panel. We believe that a great deal of research remains to be carried out before the place of fibrinolytic therapy in medical practice can be established. Investigations so far have been designed to define the situations in which fibrinolytic agents might be effective and not to evaluate the clinical usefulness of fibrinolytic therapy. These objectives are quite different. I can illustrate some of the problems which have resulted by reviewing our own experience. We were trying to find out whether or not plasmin could dissolve clots. Our final result was a collection of clinical data which could be subjected to conventional analysis and yield superficially convincing "proof" that thrombolytic therapy is useful. However, our results do not really constitute a valid evaluation of such therapy.

TABLE 1
Fibrinolytic Therapy: Clinical Experience

Category	No. of Cases
Total cases referred	350 (approx.)
Treatment refused (geographic prob- lems, clot too old, terminal patient, no drug available, etc.)	160 (approx.)
Treated	186
Clot over 5 days old	19
"Therapeutic" cases	167
Clinical evaluation, fate of clot not certain	146
Treated less than 5 days, definite evaluation of fate of clot	21

Table I summarizes our clinical experience. We had approximately 350 candidates for plasmin therapy. One hundred-sixty of these were not treated for a variety of reasons. Of those that were treated, nineteen had old thrombotic processes, in which we expected little or no therapeutic effect. These patients were accepted for therapy principally because they offered opportunities for pharmacologic studies. This left us with 167 "therapeutic cases." Of these, we have only clinical evaluation in 146 and we know definitely whether clots had or had not been dissolved in only twenty-one. Table II shows the results in our treatment cases as compared to a control group of eighty-five patients. In our treated group, we had improvement in about 60 per cent by clinical criteria, and in two-thirds of the twenty-one patients in whom the fate of the clots was definitely ascertained. In the control group, there was clinical improvement in only 30 per cent and lysis of the clot was not demonstrated in any of four definitive cases.

The impression given by these figures is that the use of fibrinolytic therapy has resulted in at least doubling the recovery rate in thrombotic disorders. It would be naive to accept this conclusion. Our treated patients constituted, in large part, a selected group and were not matched with those in the control group. The prognosis for the control group was probably not as favorable initially. Also, this was not a blind study. Both we and our patients knew that they were receiving clot-dissolving preparations. This

TABLE II
Clinical Fibrinolytic Therapy: Results in "Therapeutic"

Catgeory	No. of Cases
Treated cases	167
Improved by clinical criteria	104
Did not improve by clinical criteria	63
Clot lysis definitively demonstrated	
Persistence of clot definitively demonstrated	7
Control cases	85
Improved by clinical criteria	25
Did not improve by clinical criteria	60
Clot lysis definitively demonstrated	0
Persistence of clot definitively demonstrated	4

TABLE III
Protocol for Double-Blind Evaluation of Plasmin
Therapy in Coronary Thrombosis

- (1) Must be less than 48 hours old for acceptance
- (2) Classification

Category

A 1."Poor risk" patient,	1.Death vs. survival
anticoagulants will be used	
A 2."Poor risk" patient,	2.If survival, evaluation
anticoagulants contrain-	of residual myocardial
dicated	damage
B. "Good risk" patient,	Reversal vs. progression
early infarction pattern	of electrocardiographic
in electrocardiogram (no	infarct pattern

Criteria for Evaluation

(3) Plan of study

Q wave)

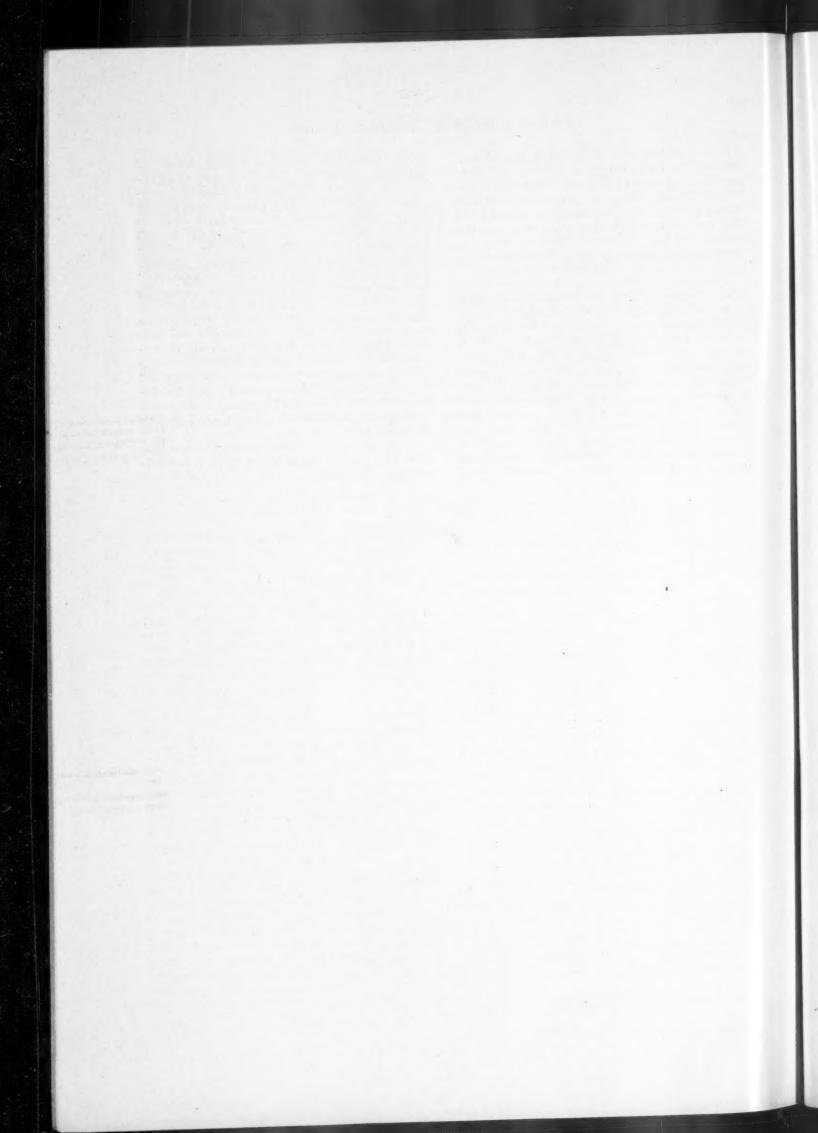
- (A) Patient is admitted by cooperating hospital and decision is made to enter him in the plasmin study
- (B) Phone call is made to the plasmin laboratory, giving the patient's name, hospital chart number, weight and diagnostic category
- (C) Plasmin technician consults a table listing the randomized allocation to treatment and control groups for each diagnostic category. He then prepares either a plasmin solution or a solution of human serum albumin of similar appearance
- (D) The treatment solution is delivered to the cooperating hospital and administered by the staff of the cooperating hospital. No member of the plasmin team sees the patient or his records
- (E) Evaluation of results is made by the staff of the cooperating hospital, who have no access to the plasmin laboratory records
- (F) Appropriate safeguards are provided to protect the patient: procedure for immediate code-break, if necessary; antiplasmin available for emergency use; etc.

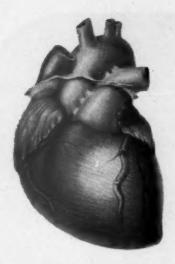
may have affected the clinical evaluation of these patients. We tried hard not to read favorable results into these cases, but I cannot guarantee that we succeeded. Finally, the treated patients received more intensive medical care than the control patients. All that we conclude from these apparently favorable statistics is that fibrinolytic therapy deserves a thorough studies must now be carried out.

The program we have initiated for coronary thrombosis is outlined in Table III. This is a double-blind study, in cooperation with a city-county hospital. The patients are admitted to the cooperating hospital, in which a clinical team decides whether or not to include a patient in the plasmin study. If the patient is included, a telephone call is placed to the Plasmin Laboratory of the Roswell Park Memorial Institute giving his name, chart number, weight and diagnostic category. The plasmin technician consults a previously prepared randomized list which assigns the patient to either a group receiving treatment or a group receiving placebo within each category. He then prepares either a solution of plasmin or one of

similar appearance containing a small amount of human serum albumin. This is delivered to the cooperating hospital. The material is infused by physicians at this hospital and the results are evaluated by them, without knowing whether plasmin or serum albumin was infused. No member of the Roswell Park plasmin team sees the patient. The results are evaluated separately for each diagnostic category. Patients are classified as "poor risks" or "good risks" by Russek's criteria. Because "good risk" patients do quite well anyway, we have excluded the ordinary patient of this type from the study. However, we accept "good risk" patients with signs of a very early lesion, where we might hope for a dramatic electrocardiographic reversal. Our evaluation criteria in the poor risk cases are (1) survival versus death and (2) residual myocardial function after the acute episode. In the "good risk" patients, evaluation is based on the speed of reversal of electrocardiographic abnormalities.

We estimate that in two to four years, we will have some idea whether fibrinolytic therapy is useful in coronary thrombosis.





IN ANGINA PECTORIS AND CORONARY INSUFFICIENCY

the treatment must go further than vasodilation alone. It should also control the patient's ever-present anxiety about his condition, since anxiety itself may bring on further attacks.



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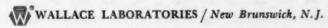
before meals and at bedtime. according to individual require-

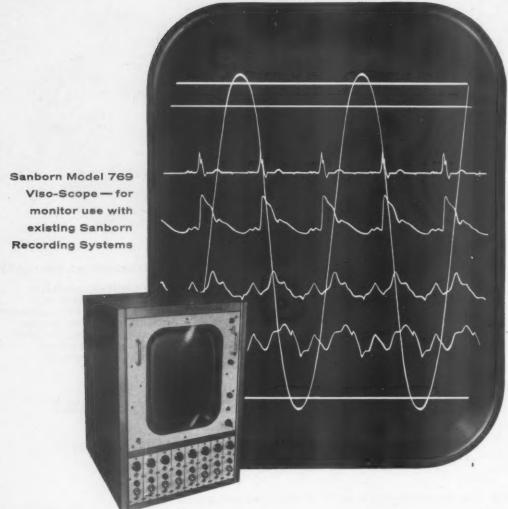
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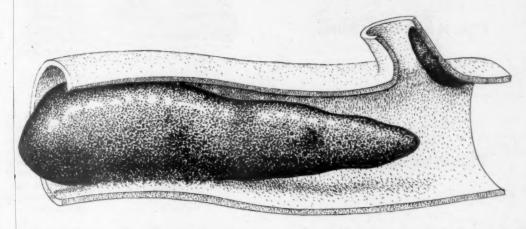
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THROMBOLYSIN, HUMAN

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Bed rest

Effect on intravascular thrombi



Clot may form permanent obstruction to blood flow. New clots may form.

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Sudden death from pulmonary embolism is an ever-present hazard. One or more nonfatal pulmonary emboli may result in irreversible lung damage or secondary pneumonia.

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Chronic leg swelling, severe secondary varicose veins, and leg ulcers are common sequelae.











Anticoagulant + Bed rest

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Anticoagulants cannot remove formed clot. However, they help prevent its extension and minimize formation of new clots.



Recently formed intravascular clots are lysed and the formation of new clots is inhibited. Circulation is restored and maintained, with rapid symptomatic relief.



The careful use of anticoagulants reduces the occurrence of pulmonary emboli.



The incidence and severity of pulmonary emboli are greatly reduced since THROMBOLYSIN acts to remove thrombi before they can become emboli.



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A striking reduction is observed in the duration of hospital stay, bed rest, and convalescence.



The incidence and severity of the postphlebitic syndrome are reduced.



Postphlebitic complications are prevented or greatly minimized.











What is THROMBOLYSIN?

Thrombolysin is Fibrinolysin, Human. It is prepared by activating the profibrinolysin-rich Fraction III — 3 of pooled human plasma with highly-purified streptokinase and then lyophilizing it. Thrombolysin helps restore the natural equilibrium between clot formation and clot lysis, thereby enhancing the ability of the blood to maintain normal flow.

In What Conditions is it Indicated?

THROMBOLYSIN is indicated in thrombophlebitis, phlebothrombosis, pulmonary embolism, and certain arterial thrombi.

*(NOTE: Successful lysis of thrombi of major cerebral vessels has been reported. However, additional experience is required to define the indications and contraindications of therapy in such patients. THROMBOLYSIN has also been administered to patients with acute myocardial infarction, but the scope of this work is still too limited to permit conclusions about its safety or benefit.)

When Should Therapy be Initiated?

Treatment with Thrombolysin should be started as soon as possible after a thrombus has formed. Blood clots begin to organize shortly after formation and may become encased in a layer of endothelial cells, making them resistant to the action of Thrombolysin. Usually, more rapid lysis can be expected to take place when treatment is initiated within five days after a thrombus has formed; however, in some cases successful lysis has been accomplished when treatment was not initiated for several weeks after thrombus formation.

Can THROMBOLYSIN be Given to Patients

Being Treated with Anticoagulants?

Yes. Patients who have been on anticoagulant therapy can be expected to improve when Thrombolysin is added to their program of treatment.

Does THROMBOLYSIN Increase the Incidence of Embolism?

Clinical studies indicate that it does not. In fact, if any evidence of embolization should appear, it is important to continue Thrombolysin until symptoms have disappeared.

What is the Dosage?

The dosage most frequently used by investigators has been 4 vials (200,000 MSD units) per day by intravenous infusion. This is usually administered by giving 1 vial per hour for 4 consecutive hours. Alternatively, 1 vial (50,000 MSD units) per hour may be given for 2 consecutive hours and repeated in 3 to 6 hours. The dosage range is 1 vial (50,000 MSD units) to 2 vials (100,000 MSD units) an hour by intravenous drip, for 1 to 6 hours, depending on the nature of the clot and the response

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of the patient. Most patients respond in one day; those who do not may require additional doses for three or four successive days.

Patients not under active treatment with anticoagulants at the time of

the thromboembolic episode:

New clot formation is unlikely to occur during the administration of Thrombolysin, so that anticoagulants may be unnecessary in this period. However, the fibrinolytic activity of Thrombolysin persists only 3 to 4 hours after cessation of infusion; in patients subject to thrombosis, provision should be made to provide adequate therapeutic anticoagulant effect at this time.

Patients under active treatment with anticoagulants:

Within recommended dosages, Thrombolysin produces only minor alterations in the clotting mechanism: the prothrombin time is generally increased by only a few seconds, the Lee-White clotting time by only 1 to 4 minutes, and the fibrinogen levels generally decrease by about 30 percent of control values. In themselves, these alterations are probably of no clinical significance. In patients on concurrent anticoagulant therapy in whom the clotting mechanism is depressed to midtherapeutic levels, the small additional depression due to Thrombolysin should produce no added danger; however, the addition of Thrombolysin may be hazardous when the therapeutic anticoagulant level already threatens to exceed safe limits.

What Other Precautions are Necessary?

THROMBOLYSIN is contraindicated in the presence of a hemorrhagic diathesis or hypofibrinogenemia. Fibrinolytic activity usually increases spontaneously for a short period after anesthesia or surgery. Therefore, THROMBOLYSIN should be used with caution because lysis of the clots at the operative site may occur.

Bleeding from open wounds or recent operative sites can occur during therapy. Usually this has been observed only in patients receiving both an anti-coagulant and Thrombolysin. In such cases, the bleeding was controlled by the use of plasma or whole blood transfusions. A specific antagonist to the anticoagulant may also be used.

What Side Effects May Occur?

Febrile reactions may occur, but these are rarely severe. When they do occur, the temperature usually rises rapidly to a peak, then returns to normal within 24 hours. In some patients, a rise in temperature above 1.5 to 2 degrees F. is accompanied by chills, nausea, vomiting, dizziness, headache, muscle pain, back pain, tachycardia, or hypotension.

How is it Supplied? 100-cc. vials containing 50,000 MSD units.

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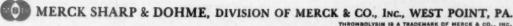
FIBRINOLYSIN, HUMAN

TO LYSE THROMBI

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TO LYSE THROMBI

TO LYSE THROMB













"Are the xanthines effective in ANGINA PECTORIS?"

(Abstract of the paper with above title)

A favorable response was unequivocally demonstrated with aminophylline when administered intravenously to angina pectoris patients. In sharp contrast the author, noted for his original contributions to cardiovascular research, found oral administration ineffective in all patients tested. This suggested that the failure was correlated with subthreshold theophylline blood-levels obtained with oral administration.

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(Russek, H. I., Am. J. Med. Sc. Feb., 1960)

CLINICAL REFERENCE DATA ON

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FORMULA: A hydro-alcoholic solution of theophylline. Each 15 cc.

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ORAL DOSAGE: First 2 days-doses of 45 cc. t.i.d. (before breakfast, at

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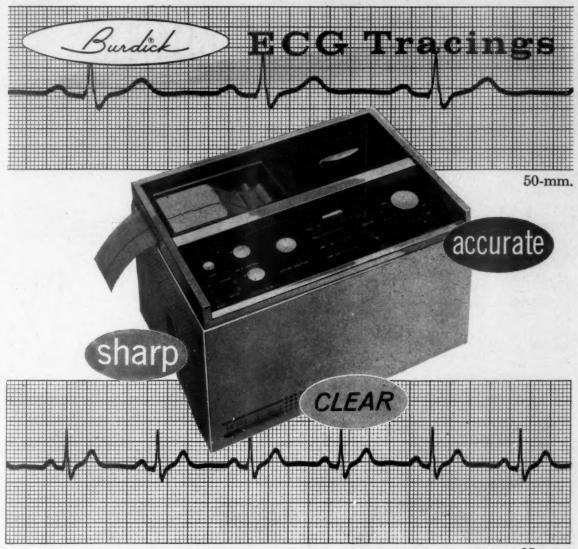
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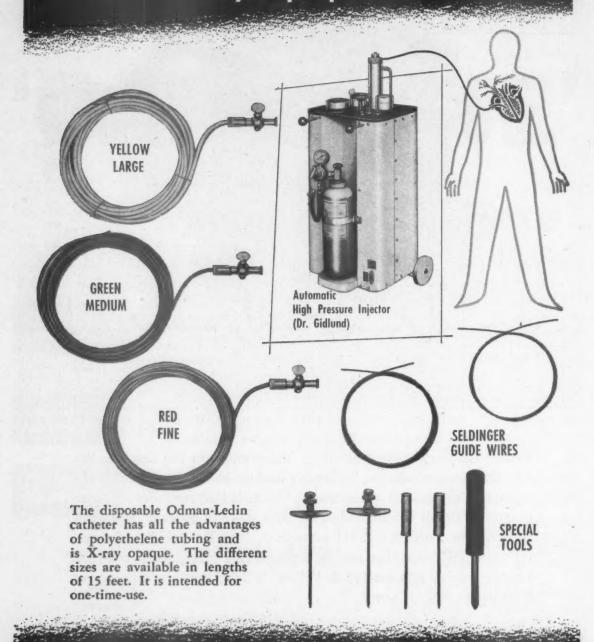
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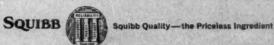
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MER/29¹⁻⁴¹ is not a cholesterol-lowering agent by the usual definition. Usual measures for lowering cholesterol only modify its intake or accelerate its metabolism. Since dietary cholesterol is the minor source of total body cholesterol, results with previous agents have been limited.

MER/29, however, inhibits cholesterol biosynthesis in the liver and other tissues. 1-4 This activity is partial and takes place at a late stage in the synthesis cycle. Sufficient cholesterol remains to fulfill its role as precursor of other necessary biosynthesized substances.

Thus MER/29 is an inhibitor of excess cholesterol production, and reduced cholesterol levels in both serum³⁻⁶ and tissues^{2.4.7} is the net result of this activity. Studies of Hollander and Chobanian,³ and those of Oaks et al.,⁶ found that cholesterol levels were lowered irrespective of diet.

In clinical studies³⁻⁶ MER/29 reduced cholesterol, on the average, 48 mg.%, and reduction ranged from 20 to 110 mg.%. Maximum reduction was reached in 5 to 8 weeks.

A report on MER/29 therapy for patients with hypercholesterolemia and its probable associated conditions:

- coronary artery disease
 (angina pectoris, postmyocardial infarction)
- · generalized atherosclerosis

In some instances,3-6 MER/29 increased exercise tolerance in patients with angina pectoris. Frequency and severity of anginal attacks were reduced, as was nitroglycerine dependence. Abnormal ECG's did not occur in these patients at the previous levels of exercise. Many of them reported improved sense of good health and well-being. Explanation of these clinical benefits is not yet known. Nevertheless, they prompt mounting interest in MER/29 as an important new agent.

It is equally important that MER/29 has been well tolerated and relatively free of toxic effects. Clinical liver damage has not been encountered; however, since the principal site of action of MER/29 is in the liver, periodic hepatic function tests may be desirable until more long-term safety data are available.

Available: Bottles of 30 pearl gray capsules.

MER/29

1. MacKenzie, R. D., and Blohm, T. R.: Fed. Proc. 18:417, 1959. 2. Blohm, T. R.: Kariya, T., and Laughlin, M. W.: Arch. Biochem. 85:245, 1959. 3. Hollander, W., and Chobanian, A. V.: Boston M. Quart. 10:37, 1959. 4. Kountz, W. B.: Proceedings, Conference on MER/29, Progr. Cardiovasc. Dis. 2:(Suppl.), 541 (May) 1960. 5. Oaks, W., and Lisan, P.: Fed. Proc. 18:428, 1959. 6. Oaks, W.; Lisan, P., and Moyer, J. H.: Arch. Int. Med. 104:527, 1959. 7. Blohm, T. R.; Kariya, T.; Laughlin, M. W., and Palopoli, F. P.: Fed. Proc. 18:369, 1959. 8-41. Additional references available on request.

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Lown, B., and Levine, S. A.: Current Concepts in Digitalis Therapy, Boston, Little, Brown & Company, 1954, p. 23, par. 2.

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all...day...long



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Side effects from PERMITIL, at the recommended dosage, have been observed infrequently or not at all. Complete information concerning the use of this drug is available on request.

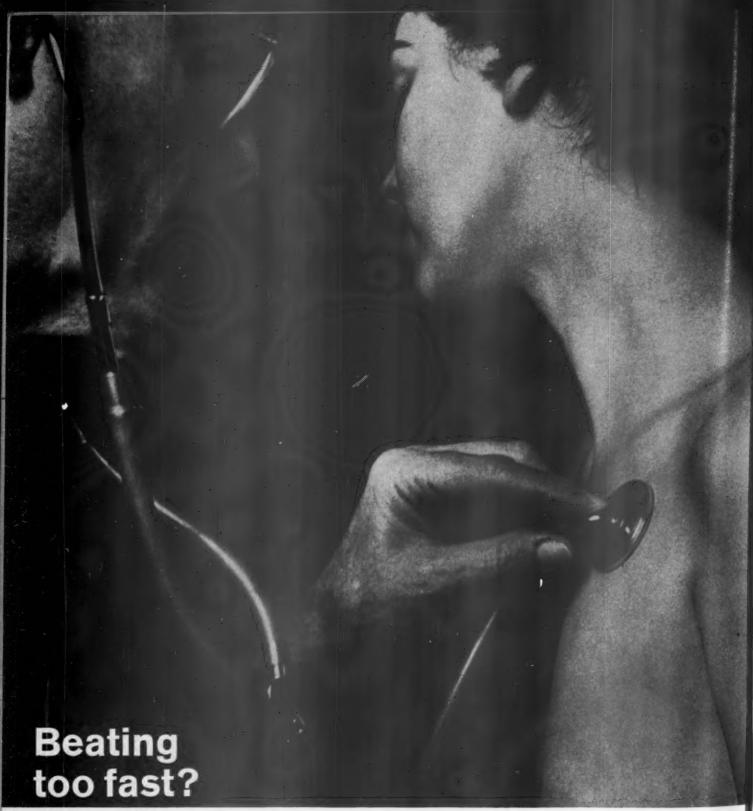
PERMITIL CHRONOTABS, 1 mg., bottles of 30. Also available, PERMITIL Tablets, 0.25 mg., bottles of 50.

References: 1. Recent compflation of case reports received by the Medical Department, White Laboratories, Inc. 2. Erast, E. M.: Clin. Med. (in press). Additional bibliography: Ayd. F. J., Jr.: Current Therap. Res. 1:41, 1959. Bodi, T., et al.: Clin. Res. 8:72, 1960. Dunlop, E.: Personal communication. Grimaldi, R.: Presented at Annual Congress of Pan-American Medical Association, May 6, 1960, Mexico City. Olson, J., and Carsley, S. H.. Personal communication.



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Slow it down with SERPASIL

Serpasil has proved effective as a heart-slowing agent in the (reserpine CIBA) following conditions: mitral disease; myocardial infarction;

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SUPPLIED: Tablets, 0.1 mg., 0.25 mg. (scored) and 1 mg. (scored). Complete information available on request.

C I B A

Danilone, Schleffelin) ®

"the anticoagulant drug of choice"2

When DANILONE was administered to 33 postmyocardial infarct patients, only two thromboembolic episodes occurred in two patients in 565 patient-months. Five such episodes in 50 patient-months occurred in four patients who had stopped taking anticoagulants.³

"twice as fast in reaching therapeutic levels" In 117 patients, the average time DANILONE required to attain therapeutic levels was less than 36 hours. With bishydroxycoumarin, two to four days are required for equal effect. 5

"a greater degree of control" DANILONE held prothrombin activity in 200 patients to between 5 and 10-per cent of normal "with greater facility" than in another series where bishydroxycoumarin was used. Generally, most satisfactory control can be obtained by dividing the dosage schedule into two 12-hour intervals. 3.6

"a reputation for safety" DANILONE, unlike the coumarin derivatives, has a short recovery period and no cumulative action. 5,8,9 As a result, DANILONE "... can be administered with reasonable safety in the presence of many conditions which would contraindicate the use of Dicoumarol."

"a lack of toxic reactions" Because of its extremely low incidence of toxicity, 2,4,9 and "... the apparent safety factor of a wide spread between therapeutic and toxic dose, it is felt that phenyldanedione [phenindione] is the anticoagulant drug of choice." 2

postcoronary prognosis
"a significant reduction" in subsequent serious infarcta!

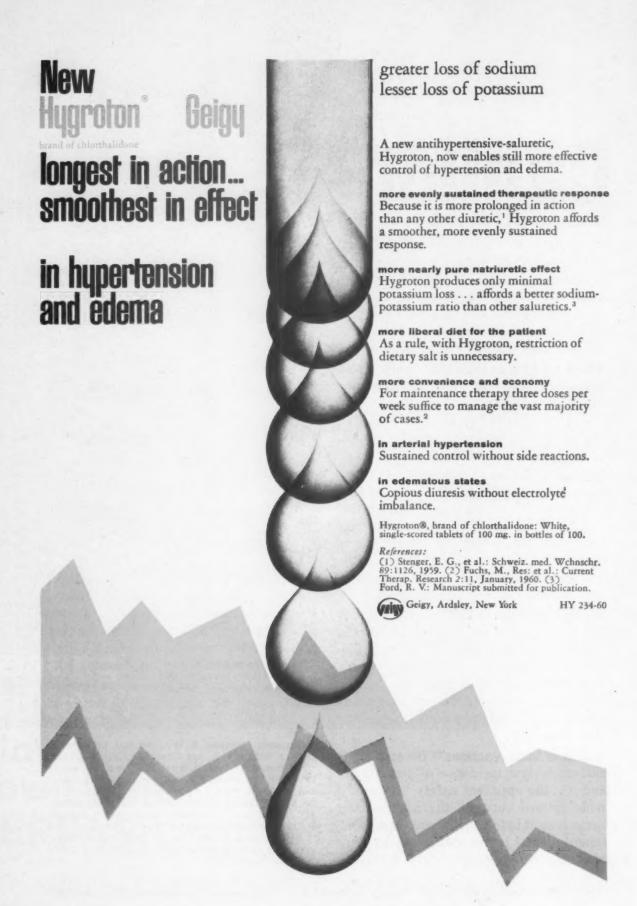
"inexpensive" DANILONE is the least expensive anticoagulant available today. Maintenance cost is as little as three cents a day. In addition, its stability of effect permits fewer prothrombin-time tests, saving the patient additional costs.

Samples and literature available on request. SUPPLIED: 50 mg. scored tablets. Bottles of 100 and 1,000.

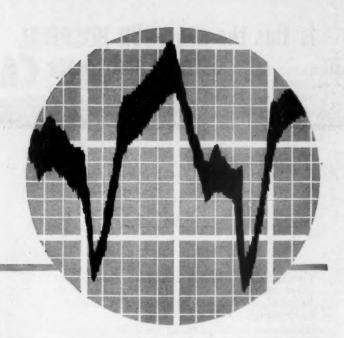
References: 1. Pickering, G., et al.: Brit. M. J. 1:803 (Mar. 28) 1959. 2. McCabe, E. S., and Fittipoldi, J., Jr.: Am. Pract. & Digest Treat. 4:765, 1953. 3. Drinan, F. W., et al.: Am. Heart J. 53:284, 1957. 4. Harper, B. F., and Johnson, R.: J.M.A. Georgia 45:149, 1956. 5. Wood, J. E.; Beckwith, J. R., and Camp, J. L., III: J.A.M.A. 159:635 (Oct. 15) 1955. 6. Sise, H. S.; Maloney, W. C., and Guttas, C. G.: Am. Heart J. 53:132, 1957. 7. Newcomb, T. F.: New England J. Med. 260:545 (Mar. 12) 1959. 8. O'Connor, W. R.; Thompson, C. E., and Baker, L. A.: Quart. Bull. Northwestern Univ. M. School 26:193 (Fall) 1952. 9. Toohey, M.: Brit. M. J. 1:650 (Mar. 21) 1953.



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QUINIDINE hydrochloride

Injectable Quinidine Hydrochloride, Brewer—the first injectable quinidine available in America—is especially indicated in ventricular tachycardia and in certain cases of auricular fibrillation. It usually begins to act within 15 to 30 minutes, and reaches its maximum effect in 1½ to 3 hours.

In one study of 107 cases of paroxysmal ventricular tachycardia, the investigators conclude: "The treatment of choice was quinidine therapy."

In refractory cases of paroxysmal tachycardia, the intravenous administration of Quinidine has proved effective.²

ADMINISTRATION: Intramuscularly, or if necessary, intravenously.

SUPPLIED: Quinidine Hydrochloride Injectable (0.6 gm.) in 5 cc. ampuls. Quinidine Hydrochloride Injectable (0.18 gm.) in 1½ cc. ampuls. Also available for oral administration—Quindul (Quinidine Sulfate, Brewer) (3 gr.) in capsules, tablets and enteric coated tablets.

Additional information and clinical reports forwarded on request

Brewes

Brewer & Company, Inc.

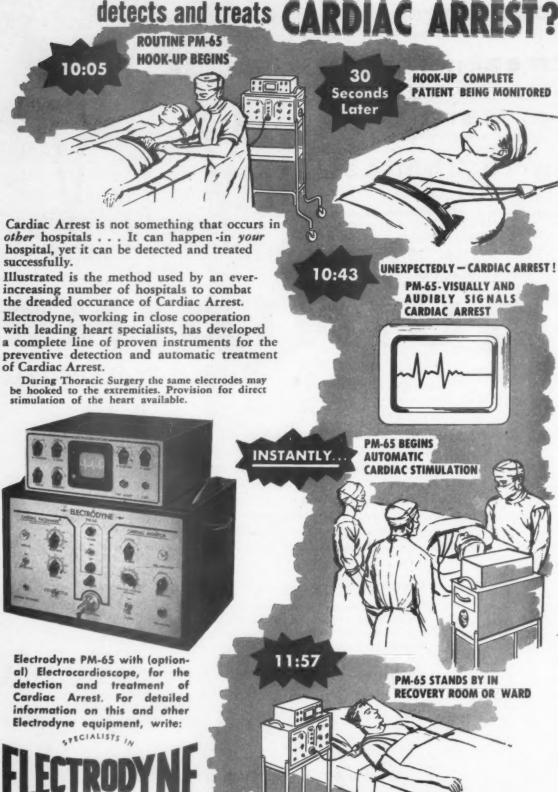
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1. Armbrust, C.A., Jr., and Levine, S.A.: Paroxysmal Ventricular Tachycardia: A Study of 107 Cases, Circulation 1:28 (1950)

 Bell, G.D., Bradley, R.B., and Hurxthal, L.M.: Paroxysmal Tachycardia, Experiences with Massive Doses of Quinidine Intravenously in a Refractory Case. Circulation 1:939 (1950)

Is this the way YOUR HOSPITAL detects and treats CARDIAC ARREST?



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in coronary insufficiency



Metamine Sustained helps you dilate the coronaries

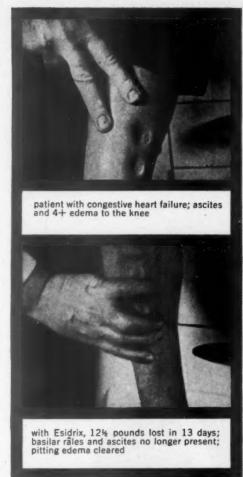


METAMINE SUSTAINED (triethanolamine trinitrate biphosphate, 10 mg., in a unique sustained-release tablet) is a potent and exceptionally well tolerated coronary vasodilator. Pharmacological studies at McGill University demonstrated that METAMINE "exerts a more prolonged and as good, if not slightly better coronary vasodilator action than nitroglycerin . . ." Work at the Pasteur Institute established that METAMINE exerts considerably less depressor effect than does nitroglycerin. Virtually free from nitrate side effects (nausea, headache, hypotension), METAMINE SUSTAINED protects many patients refractory to other cardiac nitrates, and, given b.i.d., is ideal medication for the patient with coronary insufficiency. Bottles of 50 and 500 tablets. Also: METAMINE, METAMINE WITH BUTABARBITAL, METAMINE WITH BUTABARBITAL SUSTAINED, METAMINE SUSTAINED WITH RESERPINE.

1. Melville, K. I., and Lu, F.C.: Canadian M.A.J., 65:11, 1951. 2. Bovet, D., and Nitti-Bovet, F.: Arch. Internat. de pharmacodyn. et therap., 83:367, 1946. 3. Fuller, H. L., and Kassel, L.E.: Antibiotic Med. & Clin. Therapy, 3:322, 1956.

Thos. Leeming & Co. Inc. New York 17, N. Y.

*Patent applied for



benefits in edema, benefits in hypertension plus added potassium protection

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ESIDRIX-K

New ESIDRIX-K provides all the oral diuretic-antihypertensive advantages of ESIDRIX, plus a properly proportioned potassium supplement. ESIDRIX produces marked excretion of salt and water in edematous patients, and in many hypertensive patients significantly reduces blood pressure, alone or with other antihypertensive drugs. Potassium excretion is minimal, and the built-in K supplement further helps eliminate problems due to potassium loss. Three ESIDRIX-K tablets provide potassium equivalent to one quart of fresh orange juice; ESIDRIX-K is coated to prevent gastric irritation.

Complete information sent on request.

Supplied: Esidrix-K Tablets (white, coated), each containing 25 mg. Esidrix and 500 mg. potassium chloride. Esidrix Tablets, 25 mg. (pink, scored) and 50 mg. (yellow, scored).

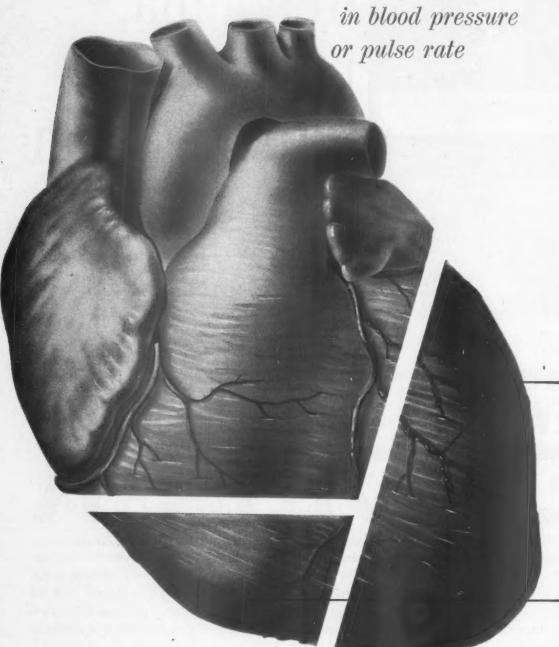
Esidrix-K is especially indicated for patients in whom even moderate potassium loss can cause complications, or those whose condition predisposes to hypokalemia. Among candidates for Esidrix-K are patients taking digitalis for congestive heart failure, those with renal or liver disease, those under long-term treatment, and those on salt-restricted diets.



ESIDRIX® (hydrochlorothiazide CIBA)

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improve coronary
blood flow with
no significant change
in blood pressure
or pulse rate



In angina pectoris, the gradual, prolonged action of Peritrate avoids significant drop in blood pressure, increase in pulse rate, and typical nitrate headache. Peritrate reduces frequency and severity of anginal attacks in 4 out of 5 patients, increases exercise tolerance, reduces nitroglycerin dependence, improves ECG findings.

In postcoronary management, gradual, prolonged action helps establish and sustain collateral circulation safely, to reduce the extent of myocardial damage, support natural healing and repair, and minimize any ensuing anginal attacks.

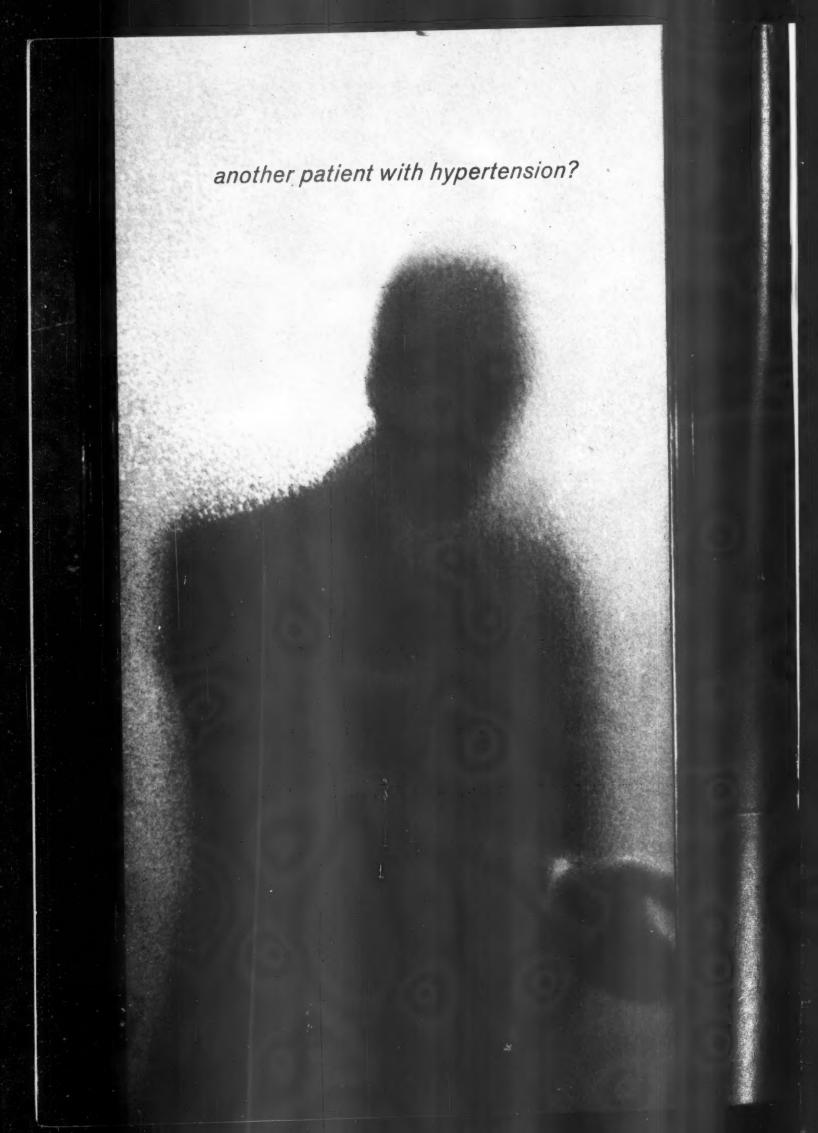
basic in coronary artery disease

Peritrate⁶

brand of pentaerythritol tetranitrate



NEW form available: Peritrate with Phenobarbital Sustained Action. 1 tablet on arising and 1 tablet 12 hours later.









indicated in all degrees of hypertension

effective by itself in most hypertensives

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HYDROPRES can be used:

- alone (In most patients, HYDROPRES is the only antihypertensive medication needed.)
- as basic therapy, adding other drugs if necessary (should other antihypertensive agents need to be added, they can be given in much lower than usual dosage so that their side effects are often strikingly reduced.)
- as replacement therapy, in patients now treated with other drugs (In patients treated with rauwolfia or its derivatives, HYDROPRES can produce a greater anti-hypertensive effect. Moreover, HYDROPRES is less likely to cause side effects characteristic of rauwolfia, since the required dosage of reserpine is usually less when given in combination with HydroDIURIL than when given alone.)

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25 mg. HydroDIURIL, 0.125 mg. reserpine. One tablet one to four times a day.

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50 mg. HydroDIURIL, 0.125 mg. reserpine. One tablet one or two times a day.

If the patient is receiving ganglion blocking drugs or hydralazine, their dosage must be cut in half when HYDROPRES is added.

For additional information, write Professional Services, Merck Sharp & Dohme, West Point, Pa.

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two reliable oral anticoagulants adapted to different needs

Sintrom® brand of acendocumarol Tromexan® brand of ethyl biscoumacetate

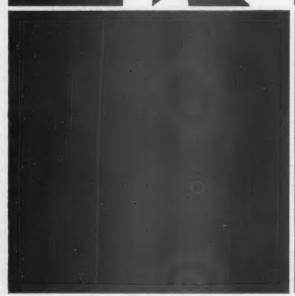
Geigy

Tromexan is distinguished by rapid onset of hypoprothrombinemic effect and by an equally rapid decline. It is particularly indicated when either prompt anticoagulant effect is imperative or termination of therapy is required, as for surgical intervention.

Sintrom has a rate of action intermediate between that of Tromexan and Dicumarol.® It is noted for the ease with which a stable prothrombin response can be maintained on uniform daily dosage. Consequently it is exceptionally well adapted to long-term therapy.

Tromexan[®], brand of ethyl biscoumacetate: Scored tablets of 150 mg. and 300 mg. Sintrom[®], brand of acenocoumarol: Double-scored tablets of 4 mg.

Geigy, Ardsley, New York





Lifts depression...as it calms anxiety!

For cardiovascular patients-a smooth, balanced action that lifts depression as it calms anxiety...rapidly and safely

Balances the mood-no "seesaw" effect of amphetamine-barbiturates and energizers. While amphetamines and energizers may stimulate the patient—they often aggravate anxiety and tension. And although amphetamine-barbiturate combinations may counteract excessive stimulation—they often deepen depression.

In contrast to such "seesaw" effects, Deprol lifts depression as it calms anxiety — both at the same time.

Acts swiftly—the patient often feels better, sleeps better, within two or three days. Unlike most other antidepressant drugs, Deprol relieves the patient quickly—often within two or three days.

Acts safely - no danger of hypotension. Deprol does not cause hypotension, tachycardia, jitteriness, or liver toxicity. It can be safely administered with basic cardiovascular therapy.

Dosage: Usual starting dose is 1 tablet q.i.d. When necessary, this may be gradually increased up to 3 tablets q.i.d. Composition: 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl) and 400 mg. meprobamate. Supplied: Bottles of 50 light-pink, scored tablets. Write for literature and samples.





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safe and practical treatment of the postcoronary patient

A basic characteristic of the postcoronary patient, whether or not cholesterol levels are elevated, is his inability to clear fat from his blood stream as rapidly as the normal subject. 1-3 Figure #1 graphically illustrates this difference in fat-clearing time by comparing atherosclerotic and normal subjects after a fat meal. 3

"Slow clearers" gradually accumulate an excess of fat in the blood stream over a period of years as each meal adds an additional burden to an already fat-laden serum. As shown in figure #2, the blood literally becomes saturated with large fat particles, presenting a dual hazard to the atherosclerotic patient: the long-term danger of deposition of these fats on the vessel walls,⁴ and the more immediate risk of high blood fat levels after a particularly heavy meal possibly precipitating acute coronary embarrassment.⁵

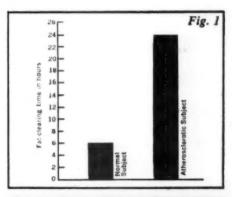
In figure #3, the test tube at the left contains lipemic serum, while the one at the right contains clear, or normal serum. If serum examined after a 12-hour fasting period presents a milky appearance, this is a strong indication that the patient clears fat slowly and is a candidate for antilipemic therapy in an effort to check a potentially serious situation.

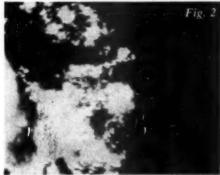
'Clarin', which is heparin in the form of a *sublingual* tablet, has been demonstrated to clear lipemic serum.^{2,6,7} Furthermore, a two-year study using matched controls resulted in a statistically significant reduction of recurrent myocardial infarction in 130 patients treated with 'Clarin'.⁸

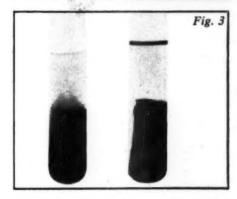
'Clarin' therapy is simple and safe, requiring no clotting-time or prothrombin determinations. Complete literature is available to physicians upon request.

References: 1. Anfinsen, C. B.: Symposium on Atherosclerosis, National Academy of Sciences, National Research Council Publication 338, 1955, p. 218. 2. Berkowitz, D.; Likoff, W., and Spitzer, J. J.: Clin. Res. 7:225 (Apr.) 1959. 3. Stutman, L. J., and George, M.: Clin. Res. 7:225 (Apr.) 1959. 4. Wilkinson, C. F., Jr.: Annals of Int. Med. 45:674 (Oct.) 1956. 5. Kuo, P. T., and Joyner, C. R., Jr.: J.A.M.A. 163:727 (March 2) 1957. 6. Fuller, H. L.: Angiology 9:11 (Oct.) 1958. 7. Shaftel, H. E., and Selman, D.: Angiology 10:131 (June) 1959. 8. Fuller, H. L.: Circulation 20:699 (Oct.) 1959.









Indication: For the management of hyperlipemia associated with atherosclerosis, especially in the postcoronary patient.

Dosage: After each meal, hold one tablet under the tongue until dissolved.

Supplied: 'Clarin' is supplied in bottles of 50 pink, sublingual tablets, each containing 1500 I.U. of heparin potassium.

*Registered trade mark. Patent applied for.

Thos. Leeming & Ca., Inc. New York 17, N. Y.

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Just two tablets



at bedtime

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... does more than lower blood pressure!

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Safety based on negligible incidence of side actions

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Practicality..simplicity of dosage ..applicable to a wide range of patients

When more potent drugs are needed, prescribe one of the convenient single-tablet combinations

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Many patients with severe hypertension can be maintained on Rauwiloid alone after desired blood pressure levels are reached with combination medication.



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